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The Colorimetric Determination of "Porphyroxine Meconidine"
The Relation between Tintometer and Spectrophotometric
Measurements obtained in the Determination of
"Porphyroxine-Meconidine" in Opiums

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In continuation of the international programme of scientific research undertaken pursuant to resolutions 159 II C (VII) and 246 F (IX) adopted by the Economic and Social Council on 3 August 1948 and 6 July 1949, respectively, the Secretary-General is presenting the following paper contributed by Canadian scientists.

The work reported here is a continuation of the narcotics research programme of the Canadian Government. The spectrophotometric data presented here were obtained along with the Lovibond tintometer readings previously reported in document E/CN.7/207, 19 October 1950. The present paper establishes a relation between the tintometer readings and the spectrophotometric readings. The measurement of the "porphyroxine-meconidine" colour is of importance to the determination of origin of seized opium.

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THE RELATION BETWEEN TINTOMETER AND SPECTROPHOTOMETRIC
MEASUREMENTS OBTAINED IN THE DETERMINATION OF
"PORPHYROXINE-MECONIDINE" IN OPIUMS

by

Charles G. Farmilo and P.M.L. Kennett

I. Purpose of the Paper

1. The purpose of the present paper is to show the relation between tintometer and spectrophotometer measurements, both used to express the relative concentrations of "porphyrroxine-meconidine" from one gramme samples of opium. "Porphyrroxine-meconidine" is determined indirectly by measuring the relative colour concentration of the red coloured material (porphyr) obtained by the treatment of "porphyrroxine-meconidine" with acid. The tintometer and spectrophotometric measurements are expressed as red units and absorbances respectively. The object of the paper is to show that a Lovibond red unit is equivalent to a definite fraction of an absorbance unit. This paper will enable chemists in other laboratories to interpret Lovibond data obtained in this series, E/CN.7/195, E/CN.7/207 and ST/SCA/SER.K/4, in terms of absorbances (optical densities) obtained at definite wave lengths of light. The samples of opium used in this study were obtained in the same way as those used previously, Farmilo and Kennett (20).

II. Experimental Procedures

2. Preparation of "Porphyrroxine-meconidine" for Spectrometry:

The determination of "porphyrroxine-meconidine" was carried out according to the method described by United Nations chemists, (See document E/CN.7/195). Opium (1.5 gm) was prepared and diluted to 60 ml. of which an aliquot (40 ml) was taken for ether extraction (representing one gramme of the original opium) and separation of ether-soluble alkaloids, including the "porphyrroxine-meconidine". The "porphyrroxine-meconidine" in the dried residue was then transformed into porphyr hydrochloride by treatment with 5 ml of 1% v/v HCl solution (1 cc concentrated HCl/100 cc water). The red coloured solution of porphyr hydrochloride was accurately diluted to 10 ml in a volumetric flask. A portion of this red solution (about 0.7 ml) was used for tintometer measurement

employing a 1/16 inch cell. Measurements published by the United Nations chemists were obtained in a 1/8 inch cell and our readings as given in the present paper should be multiplied by two, to compare with their data (17) and (18), and with the data used by Fuchs and Ullrich (9). (In E/CN.7/207 the multiplication by two was made in reporting the results (20).) For spectrometric measurements 1.5 ml of the above red solution was diluted to 25 ml in a volumetric flask. The concentration of these solutions in terms of the original weight of opium (1 gm in a 40 ml aliquot) was the same for all samples, equivalent to $(0.1 \times 1.5 \times 4) = 0.6$ gm opium per 100 ml aqueous HCl solution.

3. Instrumental Techniques Employed:

It is not proposed to describe in detail methods for determining absorption spectra, since these have been dealt with by many workers, Brode (1), Harrison and Loofburrow (2), Dufrasne (3), Friedel and Orchin (4), Twyman and Lothian (5), Mellon (14), and others (12). Hirt's "Sources of information on absorption spectrophotometry" lists the most up-to-date references, books, review articles, collections of data, etc., on the subject (21). The usual precautions were taken against solvent impurities, instrumental aberrations and in the use of the cells.

Comparison of Absorbance Curves Measured on Different Spectrophotometers:

4. Absorbances were measured in comparison with tintometer readings on three different spectrophotometers:

- (1) A Coleman Universal Spectrophotometer, Model 11, using a glass filter No. PC.4(11-214) with an optimum transmittance range from 400 to 650 m μ and a band width about 35 m μ at 511 m μ .
- (2) A Beckman Model B Spectrophotometer which is described in detail elsewhere (6) was used. The Beckman B wave length range is 320-1000 m μ with band width about 4 m μ at 520 m μ . The instrument is a rapid accurate device for routine photometric and spectrometric analyses. The model B uses a Fery prism for the light dispersion element, a new type wave length control mechanism and a new reed suspension continuously variable slit mechanism for controlling band width.
- (3) A Beckman DU quartz spectrophotometer which has a range from 210-1000 m μ and an effective band width at 520 m μ equal to 2 m μ using a slit width 0.1 mm. A description of the latest model has been given recently (6).

5. Absorbances measured by means of the three spectrophotometers referred to above are plotted against wave length in Figure 3. Curves (2) and (3) in Figure 3 were obtained using a red solution prepared from a sample of Indian opium (No. 6, Situ Mau Province) using Coleman and Beckman B instruments respectively. Curve (1) was plotted from absorbances used in constructing curve (3), making allowance for difference in cell diameter (corresponding to a 1.3 cm. cell path in both experiments). The greater increased sensitivity of the Beckman B as compared to the Coleman is illustrated.
6. Curve (4) and Curve (3) were obtained by means of the Beckman B and illustrate the slight decrease of maximum absorbance occurring on standing. The measurements plotted in Curve (4) were taken one day later than those shown in (3).
7. Two other effects which were observed generally in colorimetric determinations using the Beckman DU are illustrated by Curve (5). Inefficient extraction of "porphyroxine-meconidine" or low "porphyroxine-meconidine" content result in weakly coloured red solutions. In spite of low concentrations of red material the sharp peak with only a slight shift in wave length at which maximum absorption occurs, is observed in Curve (5).
8. It may be concluded that the narrower the measurable band width the more sensitive the instrument. Thus the most sensitive instrument is the Beckman DU and the least sensitive is the Coleman. This factor must be taken into account in establishing relative colourimetric values for "porphyroxine-meconidine" determinations.

Nomenclature and meaning of Spectrophotometric Terms Employed:

9. Although the instrumental techniques described in part 3 are well established, there is still some confusion in the literature regarding the expression of results from spectrophotometry. The terms employed in this paper conform with present usage as accepted by the United States, National Bureau of Standards (7) and (8).

10. In a recent paper Fuchs and Ullrich (5) employ the symbol $E_{1\%}^{1\text{cm}}$ for expressing their results. However, the extinction coefficient $E_{1\%}^{1\text{cm}}$ generally implies a knowledge of the concentration of the absorbing material, in this case the red substance (porphyr) formed from an undetermined amount of "porphyrroxine-mecconidine" alkaloid. The actual concentration of porphyr material in the solution is not known and therefore it is not possible at present to express an extinction coefficient. It is possible, however, to measure the absorbance (A) and to compare solutions on this relative basis, since a straight line relation between the amount of colour and the absorbance has been established. The differences in absorbances for similar solutions with different instruments, depending on band width, should also be taken into account.

III. Experimental Results

11. In Tables I, II, and III, Absorbances and Tintometer values are compared. The measurements were made on solutions prepared from the same opium, using a 1/16" cell for the Lovibond Tintometer readings. Only the red values are recorded although small values of blue and yellow were used along with the red when necessary to match the colour.

TABLE I: ABSORBANCES (OPTICAL DENSITIES) AND TINTOMETER
VALUES FOR RED SOLUTIONS FROM OPIUMS
(COLEMAN SPECTROPHOTOMETER)

Origin of Opium, Country and Province	Tintometer Reading,* Red Units	Absorbance* 1.3 cm cell	Absorbance* per Tinto- meter Unit, 1.3 cm.cell	Absorbance* per Tinto- meter Unit, 1 cm cell
<u>India</u>				
Jaora State	10.3	0.432	0.0420	0.0323
Tonk State	6.0	0.222	0.0369	0.0284
Kotah State	17.5	0.729	0.0416	0.0320
Mewar State	4.1	0.149	0.0364	0.0279
Sailana State	11.0	0.422	0.0383	0.0295
An East Punjab State	13.2	0.430	0.0328	0.0252
Dewas Jr. State	7.8	0.290	0.0372	0.0286
A Punjab Hill State	22.0	0.790	0.0359	0.0296
Piploda State	7.3	0.385	0.0526	0.0406
Export 43385	5.0	0.298	0.0596	0.0458
Export 44121	2.4	0.127	0.0522	0.0402
Gwalior State	3.1	0.142	0.0457	0.0353
Silverwalnut S.F.	3.6	0.168	0.0466	0.0359
" Seattle	5.1	0.242	0.0475	0.0365
63913 Excise	6.2	0.292	0.0471	0.0362
Seizure of Stand.	5.4	0.258	0.0478	0.0367
<u>Afghanistan</u>				
Export 63909	5.1	0.231	0.0453	0.0349
<u>Turkey</u>				
Usak No. 1	1.0	0.060	0.060	0.0462
Usak No. 2	5.1	0.198	0.039	0.0301
Corum	0.7	0.028	0.040	0.0309
<u>Yugoslavia, Export</u>				
Yugoslavia 63914	3.7	0.178	0.0483	0.0371
Yugoslavia 44125	3.6	0.158	0.0438	0.0338
Yugoslavia 63915	2.0	0.094	0.0470	0.0362
<u>Iran, Export</u>				
Iranian 63916	2.2	0.100	0.0454	0.0349

* NOTE: The Tintometer readings were obtained with half the depth of cell used by the United Nations Secretariat, and therefore must be multiplied by two if direct comparison with the U.N. values is desired. (Concerning low readings, see paragraph 12). The Absorbances were measured on solutions equivalent to 0.6 per cent opium, not 1 per cent as used by Fuchs and Ullrich.

TABLE II. ABSORBANCES (OPTICAL DENSITIES) AND TINTOMETER VALUES
FOR RED SOLUTIONS FROM OPIUMS
(BECKMAN B SPECTROPHOTOMETER)

Country of Origin of Opium	Tintometer Reading* Red Units	Absorbance* 1 cm cell	Absorbance* per Tintometer Unit, 1 cm cell
<u>India</u>			
United Provinces (Exp. 1)	17.3	0.660	0.038
(Exp. 5)	23.0	0.750	0.033
Gwalior State	12.0	0.490	0.040
Indore State	10.2	0.404	0.040
Ratlam State	13.0	0.496	0.038
Jaora State	15.1	0.611	0.041
Sita Mau State	21.7	0.830	0.038
Tonk State	10.0	0.390	0.039
Partabgarh State	15.3	0.635	0.042
Kotah State	27.2	1.049	0.039
Sailana State (Exp. 3)	14.4	0.523	0.036
(Exp. 5)	20.0	0.760	0.038
An East Punjab State	22.0	0.742	0.034
Dewas Jr. State	13.2	0.513	0.039
Piploda State	14.4	0.530	0.038
Export 43385	12.0	0.429	0.036
Export 44121	16.0	0.650	0.041
Gwalio Biscuit Opium	3.9	0.140	0.036
Silverwalnut S.F.	5.7	0.222	0.039
Silverwalnut Seattle	13.1	0.451	0.035
Excise 63913	13.2	0.450	0.034
Seizure of Standardized	11.0	0.413	0.037
<u>Afghanistan</u>			
Afghanistan 63909	6.1	0.240	0.039
Afghanistan 63913	4.0	0.160	0.040
<u>Turkey</u>			
Isparta	3.2	0.146	0.046
Afyon (presumed)	5.7	0.211	0.039
Sandikli	2.1	0.075	0.036
Malatya	4.2	0.165	0.039
Sinav	4.5	0.196	0.044
Export 44123	4.3	0.178	0.041
Export 44124	5.2	0.214	0.041
Export 63911	4.9	0.209	0.043

(This table continued on next page)

TABLE II (cont'd)

Country of Origin of Opium	Tintometer Reading* Red Units	Absorbance * 1 cm cell	Absorbance * per Tinto- meter Unit, 1 cm cell
Iran			
Export 63916	3.2	0.135	0.042
Lorestan	3.2	0.126	0.039
Isfahan	1.5	0.080	0.053
Kermanshah	0.8	0.040	0.050
Malayer (Exp. 1)	3.5	0.142	0.041
(Exp. 2)	4.0	0.145	0.036
Kerman	3.9	0.139	0.036
Khorassan	1.1	0.047	0.043
Export 63409	2.3	0.100	0.044
Stick opium, green label	3.8	0.154	0.041
Stick opium, red label	1.3	0.054	0.042
Japan 63426	29.9	1.205	0.040
(Singapore)	6.6	0.292	0.044
Mongolia 63424	4.4	0.165	0.038
Manchuria 63423	4.8	0.202	0.042
Korea	7.6	0.328	0.043

* NOTE: The Tintometer readings were obtained with half the depth of cell used by the United Nations Secretariat, and therefore must be multiplied by two if direct comparison with the U.N. values is desired.

The Absorbances were measured on solutions equivalent to 0.6 per cent opium, not 1 per cent as used by Fuchs and Ullrich.

TABLE III ABSORBANCES (OPTICAL DENSITIES) AND TINTOMETER
VALUES FOR RED SOLUTIONS FROM OPIUMS
(BECKMAN DU SPECTROPHOTOMETER)

Sample No. U.N.	Origin of Opium	Tintometer Reading* Red Units	Absorbance* 1 cm cell	Absorbance* per Tintometer Unit, 1 cm cell
1.	Turkey Aydin A	9.2 7.7	0.384 0.370	0.0417 0.0480
2.	" Afyon	9.7 8.0	0.398 0.389	0.0417 0.0486
3.	" Çorum	4.8 4.4	0.221 0.208	0.460 0.0473
4.	" Çal B	2.6 2.1	0.127 0.128	0.0488 0.0610
5.	" Usak A	3.1 3.1	0.136 0.155	0.516 0.0500
6.	" Nallihan A	11.1 10.4	0.470 0.490	0.0423 0.0471
7.	" Merzifon B	3.8 3.2	0.156 0.154	0.0411 0.0481
8.	" Burdur	5.6 5.1	0.238 0.230	0.0425 0.0451
9.	" Kula A	9.1 8.7	0.373 0.390	0.0410 0.0448
10.	" Sandikli	6.2 5.4	0.267 0.255	0.0431 0.0472
11.	" Aksehir A	5.3 3.7	0.216 0.194	0.0408 0.0524
12.	" Zile	13.8 13.0	0.569 0.570	0.0412 0.0439
13.	" Isparta A	6.2 4.5	0.240 0.226	0.0387 0.0502
14.	" Malatya	16.4 12.0	0.624 0.560	0.0381 0.0467
15.	" Export	5.7 4.1	0.244 0.205	0.0428 0.0500

(This table continued on next page)

TABLE III (cont'd)

Sample No. U.N.	Origin of Opium	Tintometer Reading* Red Units	Absorbance* 1 cm cell	Absorbance* per tintometer: Unit, 1 cm cell:
16.	Korea No. 1	1.1 0.6	0.058 0.044	0.0527 0.0733
17.	Korea No. 2	4.4 3.1	0.188 0.158	0.0427 0.0510
24.	Yugoslavia	4.5 3.8	0.186 0.178	0.0413 0.0468
25.	Greece	4.5 3.3	0.194 0.159	0.0431 0.0482
26.	Indochina (Laos) (Luang Prabang)	4.3 3.5	0.210 0.175	0.0488 0.0500
27.	Indochina (Laos) (Xieng-Khouang)	5.2 4.4	0.261 0.210	0.0502 0.0477
28.	Indochina (Vietnam) (Laokay)	7.1 6.5	0.329 0.263	0.0463 0.0405
34.	India (Uttar Pradesh)	12.2 11.5	0.578 0.520	0.0478 0.0452
35.	India (Malwa) (Madhya Bharat)	11.0 10.3	0.530 0.477	0.0482 0.0463
36.	India (Excise opium)	10.0 10.4	0.484 0.452	0.0484 0.0435
37.	China (Taiwan)	3.6 4.0	0.203 0.193	0.0564 0.0483

* NOTE: The Tintometer readings were obtained with half the depth of cell used by the United Nations Secretariat, and therefore must be multiplied by two if direct comparison with the U.N. values is desired.

The Absorbances were measured on solutions equivalent to 0.6 per cent opium, not 1 per cent as used by Fuchs and Ullrich.

The Tables list the absorbances observed using three different instruments; Table I, Coleman; Table II, Beckman B; Table III, Beckman DU. The observed absorbances for the Coleman (Table I) were obtained using a 1.3 cm. cell and are expressed in the last column as absorbances (A) per Tintometer unit (L) for a 1 cm. cell to correspond with the data in other Tables, as follows:

$$\frac{A}{L} \times \frac{1}{1.3} = A/\text{Tintometer Unit}/1 \text{ cm light path.}$$

For example, for India, Jacra State, $A/\text{Tintometer Unit}/1 \text{ cm} =$

$$\frac{0.432}{10.3} \times \frac{1}{1.3} = 0.0323, \text{ where } A = \text{absorbance, } L = \text{Tintometer Unit.}$$

12. If the Tintometer values are compared, it will be noted that those in Table I are much lower than those in Table II, for the same samples. The values in Table I are the result of early experiments in extracting the "porphyrroxine-medonidine", and are too low. However, this does not affect the relation between the Tintometer values and the Absorbances, since the latter were obtained on dilutions of these same solutions, as described in paragraph 2. These early results have therefore been used to determine the relation between Tintometer values and Absorbances for the Coleman instrument.

13. The average absorbances per Tintometer unit were obtained from Tables I, II, and III.

Table No.	Instrument	Average Absorbance per 1 cm per Tintometer Unit
III	Beckman DU	0.04688
II	Beckman B	0.03976
I	Coleman	0.03428

Concentration of opium, 0.6 per cent.

Comparison of Absorbances/Tintometer Unit with Results of Fuchs and Ullrich (5)

14. Our Tintometer results for direct comparison with those of the United Nations Secretariat (17) (18) and Fuchs and Ullrich (9) should be multiplied by two. The relationship, Absorbance per Tintometer unit, based on a 1% concentration with respect to opium as used by Fuchs and Ullrich (who compared their absorbances with the United Nations Tintometer values determined in New York), may be calculated using the Beckman DU average absorbance value, Table III, as follows: $\frac{0.04688}{6} \times \frac{10}{2} = 0.03906$. The average absorbance

per Tintometer unit for 1.0 per cent opium solution as measured by the Beckman DU is therefore 0.03906. It should be pointed out that our spectral maxima were measured at a wave length 511 m μ , whereas Fuchs and Ullrich (9) made their measurement at 520 m μ . Furthermore, they did not have direct comparisons with their own Tintometer values, as we did.

15. The comparison of values on the basis of 1 per cent opium equivalent, is as follows:

<u>Instrument</u>	<u>Observed Absorbances per Tintometer Unit</u>	
	<u>at 511 mμ</u>	<u>Fuchs and Ullrich</u>
Beckman DU	0.03906	0.0286 at 520 m μ
Beckman B	0.03313	--
Coleman	0.02856	--
Pulfrich	--	0.0253 at 530 m μ

Stability of the Red Colour in Solution

16. An important factor to be determined in any colourimetric method is the stability of the colour used for spectrophotometry. Analysis of the spectral curves (Fig. 1 and 2) of the red coloured solutions showed three maxima corresponding to absorption in the near ultraviolet at 285 m μ (Fig. 1) and 380 m μ (Fig. 2) and the middle visible region at 510 to 520 m μ (green) (Fig. 2). Table IV illustrates the measurements of absorbances on three solutions subjected to different conditions over a period of time; after heating, standing at room temperature in the dark, and exposed to daylight. The absorption maxima shift and the absorbances increase or decrease by several units depending on conditions, such as, temperature, light, "pH" and type of ions present in solution. Not all these factors were examined; however, it has been established that the porphyr colour is fairly stable to temperature and light changes. The solution used for these experiments was prepared from samples of Indian opium No. 19 (Gwalior State, Biscuit). The measurements were made in the usual way spectrophotometrically at timed intervals.

17. The coloured solutions are remarkably stable in the dark and daylight at room temperature, and also at elevated temperatures. They are eminently

suitable for colourimetric determinations. The peak at 380 m μ (Fig. 2) may represent either an absorption of the porphyr molecule, or of a transformation product from the porphyr molecule. The latter is probably the case, since it is observed that as the maximum at 510 m μ decreases in intensity with temperature or by other means, the maximum at 380 m μ increases. This also suggests the formation of a new product from the porphyr compound. Regardless of the meaning or interpretation of these results they do illustrate the important fact that the red coloured solutions are sufficiently stable for establishing a spectrophotometric method of analysis.

TABLE IV

COLOR STABILITY OF PORPHYR HYDROCHLORIDE
SOLUTIONS FROM INDIAN OPIUMS AT VARIOUS
TEMPERATURES IN DAYLIGHT AND DARK

Sample No.	Wave Length, m μ	Absorbance at various Times, Hours				Experimental Conditions
18		1	8	16	69	Hours
	390	0.080	0.079	--	0.090	) 80° C.
	510	0.127	0.122	0.119	0.108	) Daylight
19		1	7	15	65	Hours
	380	0.140	0.138	0.138	0.170	25° C.
	510	0.142	0.138	0.137	0.128	Daylight
20		1	10	--	67	Hours
	380	0.140	0.131	--	0.140	25° C.
	510	0.168	0.168	--	0.160	Dark

Comparison of Tintometer and Spectrophotometric Data:

18. By means of the Tintometer coloured solutions were matched by mixing light from three properly chosen primaries, red, yellow, and blue. Tintometer readings thus obtained for each sample of opium can be related to the spectrophotometric curve (Figures 2 and 3) in two ways. First the relationship can be established by means of tristimulus values X, Y, and Z. It is possible to calculate these by means of tables (11, 12), a set of tristimulus values corresponding to each wave length on a spectrophotometric curve, (e.g. Figure 2) at 5 m μ intervals. A spectrophotometric analysis of the Lovibond colour system was made by Gibson and Harris (15). Tintometer Ltd., makers of the Lovibond glasses have computed from the published spectrophotometric measurements the chromaticity co-ordinates x, y of all the colours of the Lovibond system (16). The "Rothamsted Odurator Vane" enables the operator to convert directly from Lovibond to C.E.I values (22). The fundamental principles involved in such calculations have been described elsewhere (13, 17).

19. A second method of relating spectrophotometric absorption data with colourimetric data is to establish the relationship by plotting the absorbance at a chosen wave length against the Tintometer scale readings. The results are scattered about the solid straight line shown in Figure 4. The straight line was obtained using the Beckman data shown in Table III, plotted against the Tintometer readings on the same samples. There appears to be a wider scattering of the points at higher concentration of porphyr hydrochloride. It should be noted that the line shown in Figure 4 represents a correlation between the transmitted red colour (observed) and the absorbed (green) colour of light passed through the red coloured solutions. The dotted line (Figure 4) represents the "best-fit" straight line calculated by Mr. T. Kelly of this laboratory from the data in Table III by the method of least squares. The dotted line (calculated) in Figure 4 falls slightly below the solid line fitted by eye. The fitted line also intercepts the (A) absorbance axis, whereas the solid line passes through the origin. The equations of the "best-fit" line are

$$A = 0.02264 + 0.04168 T$$

$$\text{or } T = -0.35795 + 23.3585 A$$

depending on whether Absorbance (A) is expressed in terms of Tintometer (T) values or vice versa.

20. The data from Dr. Fuchs' paper and the results in Tables I, II, and III were submitted for calculation of a statistical correlation co-efficient to Mr. T. Kelly, Biometrics Section of this laboratory. The results of the calculations are shown in Table V. The high correlation co-efficients, greater than 0.9 in all sets of data (maximum = 1), show that the linear relationship between Lovibond and Absorbance values is significant and is uniform.

TABLE V. CALCULATION OF CORRELATION CO-EFFICIENTS
FOR LOVIBOND-ABSORBANCE VALUES

Instrument	Set of Data	No. of Observations N	Correlation Co-efficient r	Regression Line Parameters	
				Slope b	Intercept a
Coleman	Table I	34	.989	.03819	.02219
Beckman	Table II	48	.994	.03719	.01162
Beckman DU	Table III	52	.987	.04168	.02264
Pulfrich	16 samples	16	.997	.02583	.01108
Pulfrich	10 "	11	.961	.01681	-.3044
(data left out of consideration for final relation)					
Pulfrich	All Data	27	.956	.01998	- 3.4264

Conclusions:

21. The red coloured solutions obtained have three absorption maxima, at 285 m μ , 380 m μ , and 510-520 m μ , but only the last one is certainly due to the porphyr hydrochloride.
22. Porphyr hydrochloride concentration can be estimated at 510-520 m μ . The ultraviolet peaks appear to represent two other compounds which could be estimated simultaneously.
23. A relation between the Absorbance and the Tintometer reading was established for the Beckman DU, Beckman B, and Coleman spectrophotometers. These are 0.0391, 0.0331 and 0.0286 absorbance per Tintometer unit for the Beckman DU, B, and the Coleman respectively. The last value corresponds fairly well with a preliminary value obtained by the United Nations Secretariat (19) (about 0.030). (The solution representing the colour developed from 1 gm opium and in 100 ml solution in each case.)
24. The stability of the porphyr hydrochloride solutions has been studied, and only minor changes over a long period of time were observed. The solutions are remarkably stable in colour.
25. A high correlation between Absorbance and Lovibond values was obtained. The correlation co-efficient for our data is 0.98.

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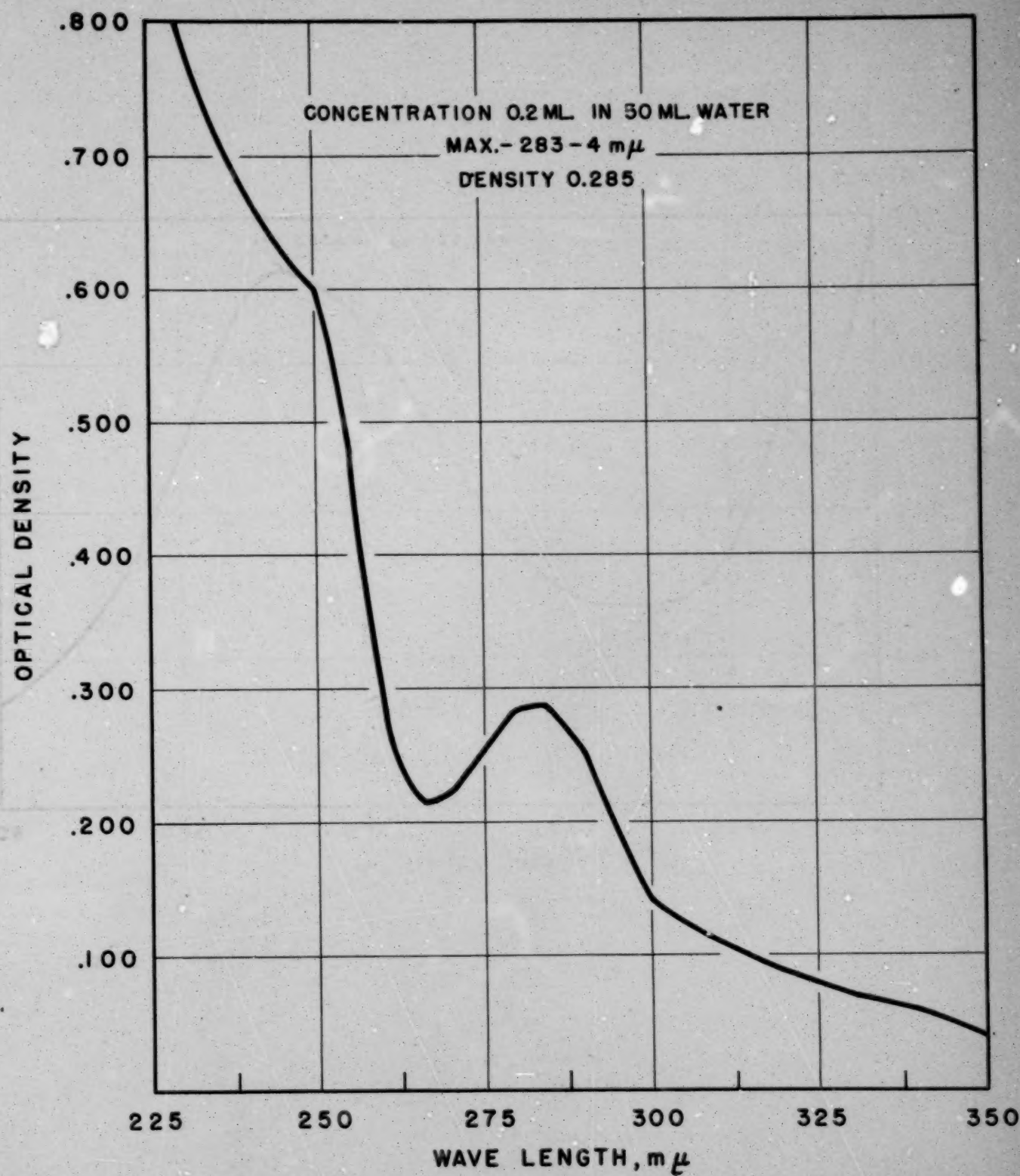


Fig. 1. ABSORPTION SPECTRUM (ULTRA VIOLET) OF SUBSTANCE PRESENT IN "PORPHYROXINE-IRON(III)" EXTRACTS

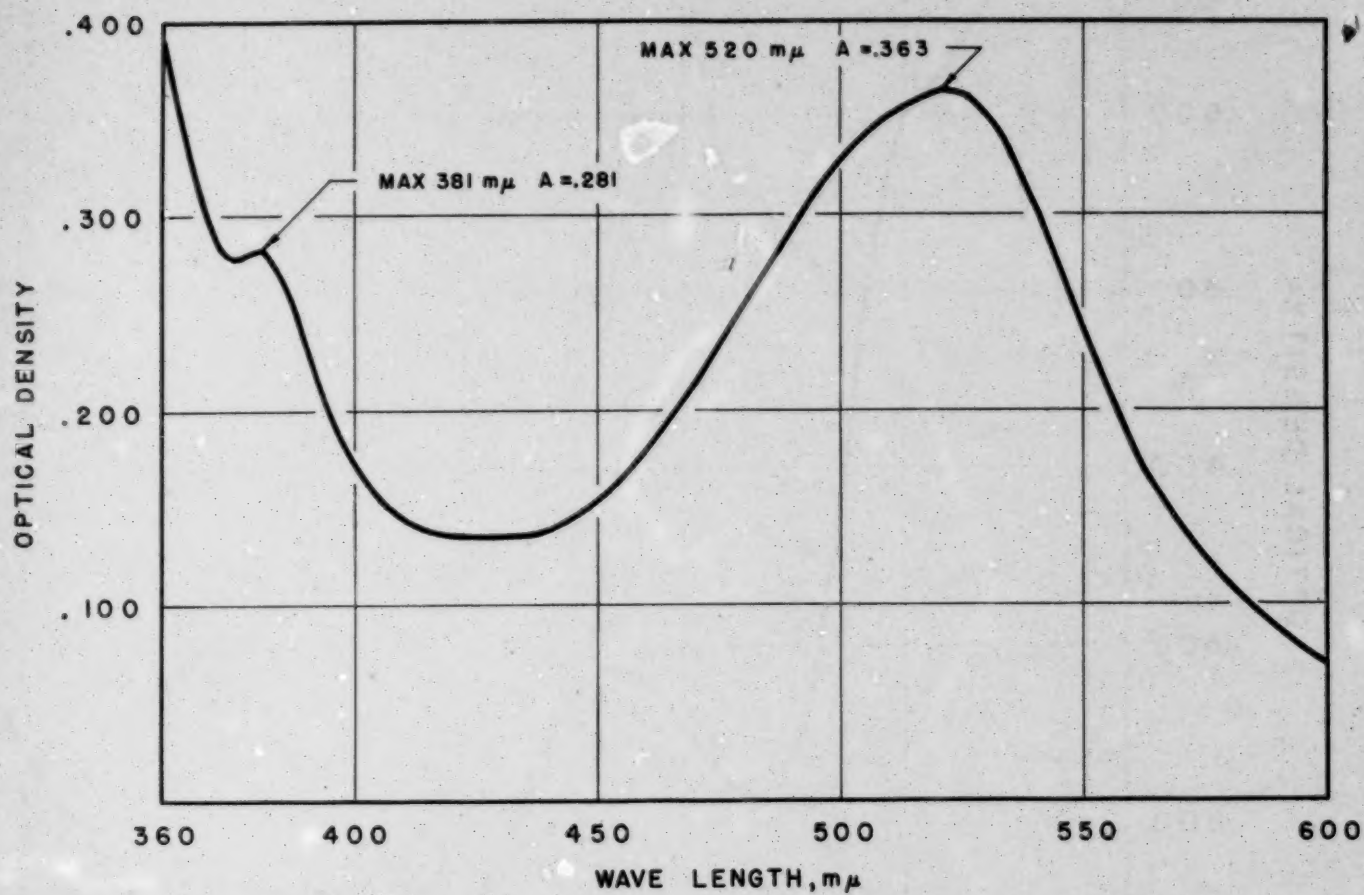


FIG. 2. ABSORPTION SPECTRUM (ULTRA VIOLET AND VISIBLE) OF PORPHYR HCL SOLUTIONS FROM OPIUM

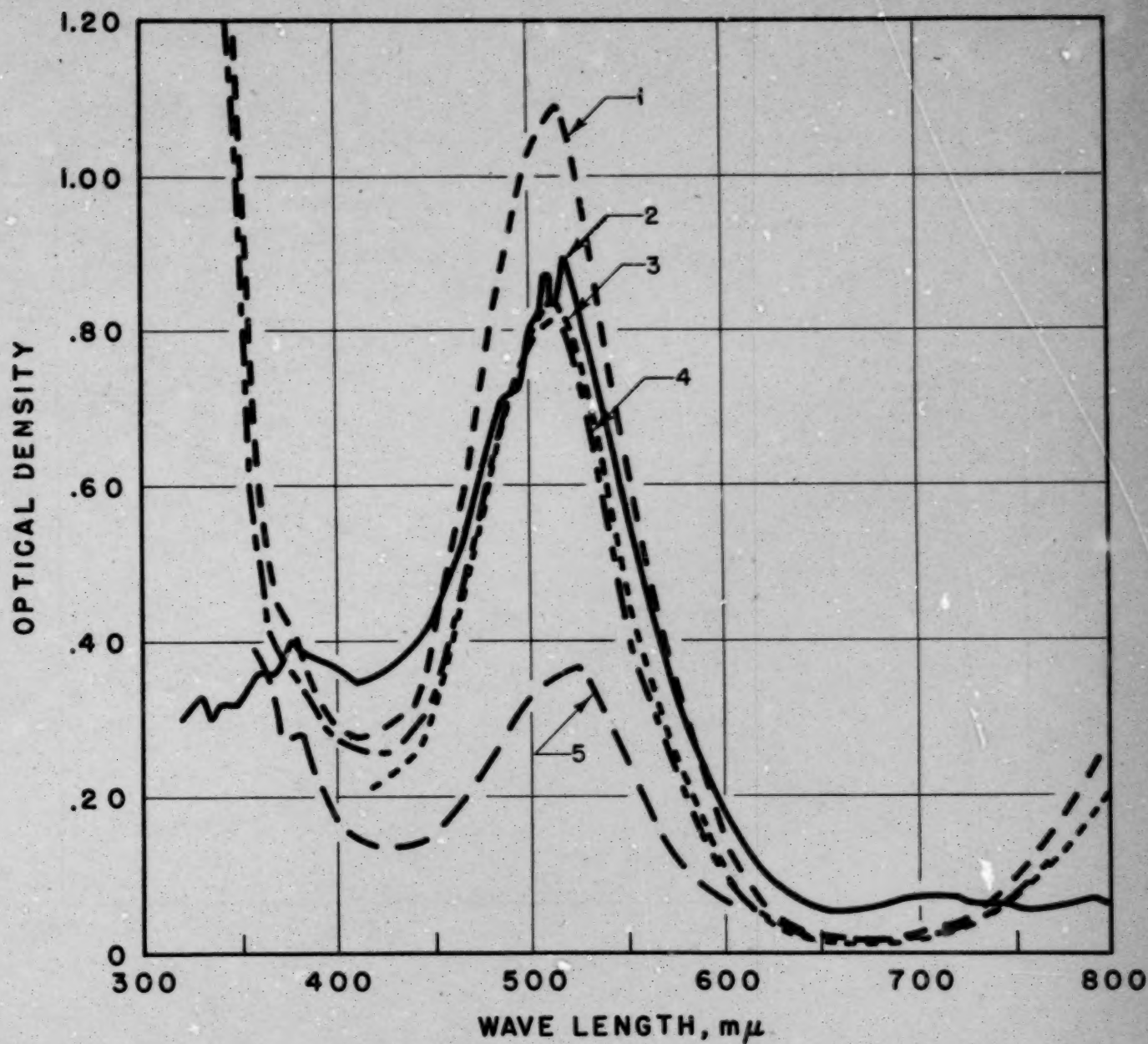


Fig. 3. COMPARISON OF ABSORPTION SPECTRA OF PORPHYR HCL SOLUTIONS MEASURED WITH THREE SPECTROPHOTOMETERS

- CURVE 1 - BECKMAN B USING 1.3 CM CELL
 - CURVE 2 - COLEMAN USING 1.3 CM CELL
 - CURVE 3 - BECKMAN B USING 1.0 CM CELL
 - CURVE 4 - BECKMAN B USING 1.0 CM CELL (Decrease of absorbance on standing)
 - CURVE 5 - LOW PORPHYR CONCENTRATION (BECKMAN DU)
- } SAME PORPHYR CONCENTRATION

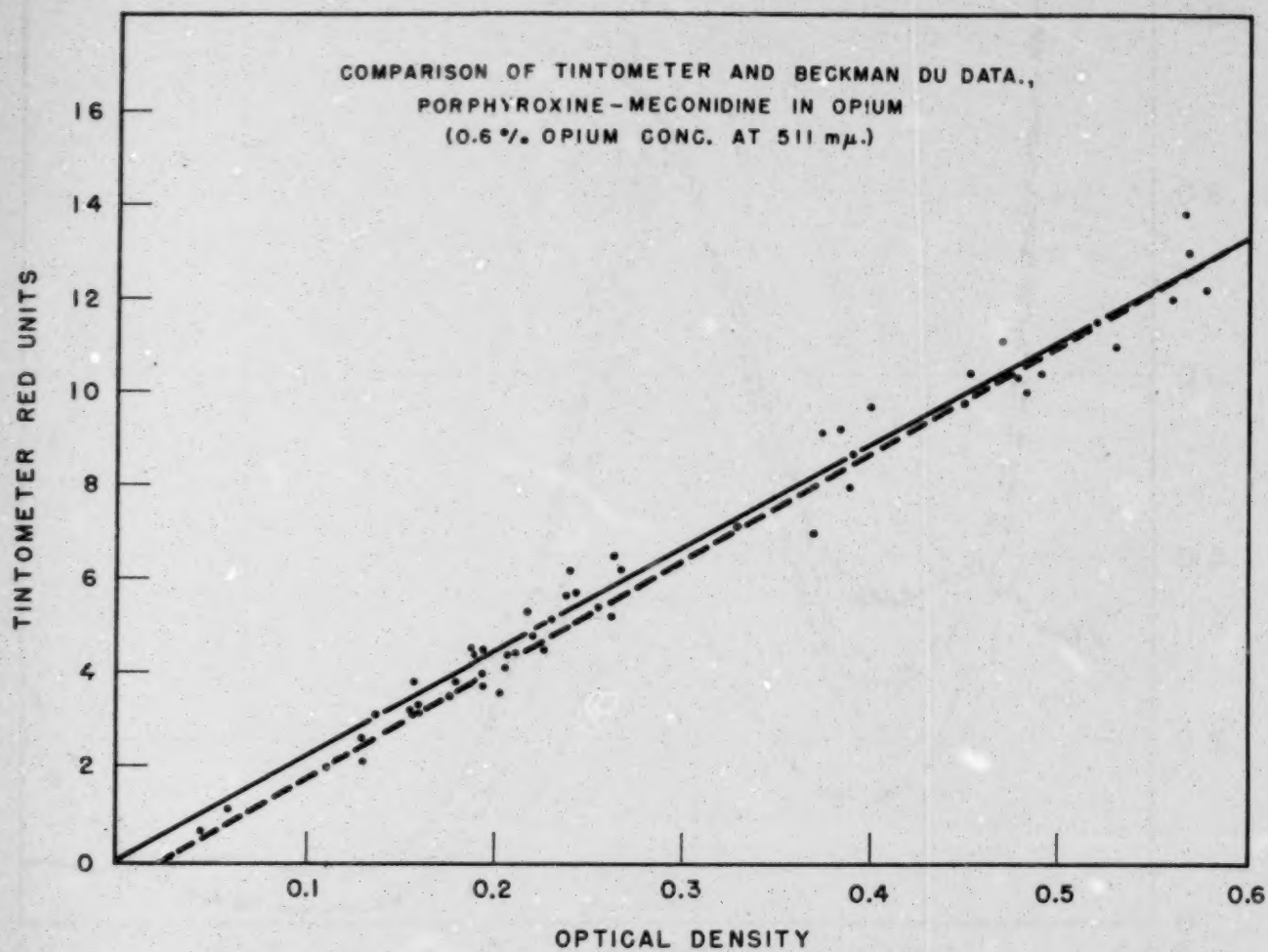


Fig. 4. RELATIONSHIP BETWEEN ABSORBANCE (OPTICAL DENSITY) MEASURED AT 511 m μ (GREEN)
AND (LOVIBOND) TINTOMETER VALUES (RED COLOR ONLY)

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THE ASSAY, CHARACTERISTICS, COMPOSITION, AND ORIGIN OF OPIUM

No. 15

An Assay Procedure for Opium based on that of the Japanese Pharmacopoeia, and morphine percentages obtained on samples from various countries

By Haruyo Asahina, Chief, Narcotic Section,
National Hygienic Laboratory, Tokyo, Japan

In continuation of the international programme of scientific research undertaken pursuant to resolutions 159 II C (VII) and 246 F (IX) adopted by the Economic and Social Council on 3 August 1948 and 6 July 1949 respectively, the Secretary-General is presenting the following paper by the Chief of the Narcotic Section of the National Hygienic Laboratory of Japan, as a contribution to the study of the problems of the opium assay and determination of the origin of opium.

53-06380

AN ASSAY PROCEDURE FOR OPIUM BASED ON THAT OF THE JAPANESE
PHARMACOPOEIA, AND MORPHINE PERCENTAGES OBTAINED ON SAMPLES
FROM VARIOUS COUNTRIES

By Haruyo Asahira

Purpose of the paper

1. The purpose of the present paper is to contribute to the study of the problems of assaying opium and determining its origin, by presenting the author's modification of the method of the Japanese Pharmacopoeia, and the results obtained on opium from various countries.

Method

2. The basic method is that of the Pharmacopoeia of Japan VI (English edition Tokyo 1952). However, there are two errors in this version, which are corrected below.
3. Another reference is the "Method of Assaying the Content of Morphine in Opium", Bulletin of the Health Organisation of the League of Nations, volume VII, extract No. 6 (1938).
4. The method of the Japanese Pharmacopoeia VI is primarily intended for standardizing powdered medicinal opium. It is a precipitation method which, like that of the United States Pharmacopoeia, does not employ any added correction. In studying it, some slight changes seemed desirable, and these are incorporated in the procedure as given below, and referred to in the notes (paragraphs 11 to 21).

Procedure for the Assay of Powdered Opium

5. Weigh accurately a quantity of 5 g. of powdered opium which has been passed through a number 5 standard mesh sieve.^{(1)*} Transfer this accurately weighed sample into a porcelain mortar with pouring lip, add 10 cc of water and triturate the mixture well for five minutes. After the mixture has been worked into a uniform paste, add 2 g. of calcium hydroxide (fresh and more

* Numbers in parentheses refer to the notes, paragraphs 11 to 21.

than 85 per cent purity)⁽²⁾ and 40 cc. of water,⁽³⁾ agitate for twenty minutes and filters.⁽⁴⁾

6. To about 30 cc. of the filtrate, add 0.1 g. of magnesium sulfate and digest for one minute by shaking, then add 0.3 g. of calcium hydroxide and digest for one minute in the same way. Allow this mixture to stand for one hour and then filter.

7. Measure accurately 20 cc. of the filtrate into a flask,⁽⁵⁾ add 10 cc. of ether and 0.2 g. of ammonium chloride, and stopper the flask. Shake the mixture two or three times, diminish the pressure in the flask by removing the stopper, and then shake vigorously by hand. As soon as crystals separate from the solution, place the flask on a mechanical shaker for thirty minutes. Remove and allow to stand overnight.⁽⁶⁾

8. The following morning cautiously pour off the ether layer and then the aqueous layer onto a quantitative filter paper of 7 cm. diameter.⁽⁷⁾ Rinse the crystals adhering to the inner wall of the flask with three successive 5 cc. portions of water saturated with ether. Each washing should be poured onto the filter paper so as to wash the filter paper and crystals thereon.⁽⁸⁾ Finally wash out the mouth of the flask and upper part of the filter paper with 5 cc. of etherized water.⁽⁹⁾

9. Transfer the filter paper with crystals to a beaker. Completely dissolve the crystals in the flask with 15 cc. of N/10 HCl, and pour the solution into the beaker containing the filter paper. Wash the flask four times with 5 cc. portions of water.⁽¹⁰⁾ and transfer all the washings to the same beaker. Titrate the excess of acid with N/10 potassium hydroxide solution. (two drops of methyl red T.S. as indicator). The end point is determined after five minutes standing.

1 cc. of N/10 HCl consumed = 28.53 mg. of $C_{17}H_{19}O_3N$ ⁽¹¹⁾

10. When ferric chloride T.S. is cautiously added along the wall of the beaker the neutralized solution should produce a greenish blue colour (test for morphine).

NOTES

11. Note 1: In the League of Nations procedure, the degree of pulverization of "hard opium" is so regulated that the powder will all pass through a 0.3 mm. sieve (IV. Homogenisation of samples).
12. Note 2: The English version of the Japanese Pharmacopoeia erroneously specifies 2 g. of potassium hydroxide instead of calcium hydroxide at this point.
13. Note 3: The total quantity of water should not exceed but be just 50 cc. during the complete procedure. A burette should be used to facilitate accurate measurements. Water and calcium hydroxide should be added gradually. Purity for the calcium hydroxide of better than 85 per cent is required by the League of Nations method. The purity should be tested as prescribed by the League method.
14. Note 4: The opinion is that it is not absolutely necessary to dry the filter paper, as prescribed by the Japanese Pharmacopoeia. It should be noted that the filter paper should not be too large for 20 cc. of filtrate.
15. Note 5: The filtrate should be clear. If turbid, results may be high; therefore if turbidity is observable in the course of the filtration the solution should be refiltered with a new filter paper. A 20 cc. volumetric pipette is used for accurate measurement of the filtrate. The use of a shaking flask as container for the 20 cc. filtrate is recommended.
16. Note 6: The stage at which the crystals separate out after the inner pressure is diminished is most important for the success of the assay. Vigorous shaking makes fine crystals separate out easily. In case of the "powdered opium", the length of time of standing prescribed by the Japanese Pharmacopoeia is twenty-four hours, for "opium alkaloids hydrochlorides", twelve to eighteen hours. However, for practical reasons, in the present modified procedure it is made just "overnight". Furthermore it is decided that "a cool place", as specified by the Pharmacopoeia, is not necessary.

17. Note 7: The filter paper is folded in four and placed in a funnel larger than the paper so as to leave about 2 mm. space above the filter paper.
18. Note 8: The phrase "the small amount of" in the Japanese Pharmacopoeia is deleted ("the small amount of crystals on the filter").
19. Note 9: The last washing with 5 cc. ether-saturated water is principally for removal of any colouring matter and acid-consuming impurities adhering to the upper part of the filter paper.
20. Note 10: This revised procedure follows that given for "opium alkaloids hydrochlorides" in the Japanese Pharmacopoeia.
21. Note 11: The procedure in the Japanese Pharmacopoeia refers to "each cc. of tenth-normal potassium hydroxide" instead of "each cc. of acid consumed" as equivalent to 28.53 mg. of morphine.

22. Analytical results

Opium samples

Loss in weight on drying (per cent)	Morphine content in per cent			
	as determined		on dry basis	
	I	II	I	II

1. Turkey U.N. 1, Aydin D	9.36	15.62	15.55	17.23	17.16
2. Turkey U.N. 2, Afyon B	9.30	12.25	12.22	13.51	13.47
3. Turkey U.N. 3, Corum C	8.72	17.60	17.45	19.28	19.12
4. Turkey U.N. 4, Cal C	7.38	14.50	14.38	16.00	15.87
5. Turkey U.N. 5, Usak D	9.73	14.14	14.09	15.66	15.61
6. Turkey U.N. 6, Nallihan C	8.41	9.10	9.09	9.94	9.92
7. Turkey U.N. 7, Merzifon D	7.80	15.49	15.33	16.80	16.63
8. Turkey U.N. 8, Burdur B	6.77	13.70	13.70	14.69	14.69
9. Turkey U.N. 9, Kula B	7.36	13.85	13.76	14.95	14.85
10. Turkey U.N. 10, Sandikli C	7.48	11.73	11.67	12.68	12.60
11. Turkey U.N. 11, Aksehir B	7.81	12.03	12.15	13.05	13.18
12. Turkey U.N. 12, Zile D	7.84	13.75	13.76	14.92	14.93
13. Turkey U.N. 13, Isparta B	8.79	10.33	10.52	11.33	11.53

Opium samples	Loss in weight on drying (per cent)	Morphine content in per cent			
		as determined		on dry basis	
		I	II	I	II
14. Turkey U.N. 14, Malatya D	7.80	16.41	16.17	17.80	17.54
15. Turkey U.N. 15, Export	6.83	13.62	13.60	14.62*	14.60*
16. Iran U.N. 52, Nehavand	10.19	9.34	9.38	10.40	10.44
17. Iran U.N. 49, Kerman	11.47	10.35	10.45	11.69	11.80
18. Iran U.N. 47, Fars	10.06	11.35	11.08	12.62	12.32
19. Iran U.N. 51, Khorassan	11.32	9.79	9.94	11.04	11.21
20. Iran U.N. 50, Kermanshah	10.11	10.64	10.47	11.84	11.65
21. Iran U.N. 48, Isfahan	10.51	11.25	11.00	12.57	12.29
22. India U.N. 36, Excise	11.65	9.98	9.97	11.30	11.28
23. India U.N. 34, Banaras	9.38	10.23	9.97	11.29	11.00
24. India U.N. 35, Malwa	10.53	10.69	10.63	11.95	11.88
25. Vietnam U.N. 28, Laokay, Tonkin	7.97	11.45	11.44	12.44	12.43
26. Laos U.N. 26, Luang Prabang B	9.42	6.91	6.86	7.62	7.57
27. Laos U.N. 27, Xieng Khouang B	8.78	12.15	12.38	13.32	13.57
28. Yugoslavia No.1, U.N. 24	8.93	16.26	16.30	17.85	17.90
29. Yugoslavia No. 2, U.N. 38	9.60	15.68	15.59	17.35	17.25
30. Korea No. 1, U.N. 16	10.56	2.54	2.63	2.84	2.94
31. Korea No. 2, U.N. 17	8.87	7.81	7.89	8.57	8.66
32. China, U.N. 37 B	7.60	7.73	7.85	8.37	8.50
33. Greece, U.N. 25 G	8.29	15.84	15.86	17.27	17.29
The remaining samples were opiums available in Japan					
34. Turkey	8.56	13.67	13.74	14.95	15.03
35. China (Manchuria)	8.35	11.05	10.97	12.06	11.97
36. Mongolia	9.04	10.34	10.10	11.37	11.10
37. Korea	9.60	11.16	11.06	12.35	12.23
38. China	13.12	6.20	6.19	7.13	7.12
39. Japan	9.55	10.27	10.31	11.35	11.40

* Note by the Secretariat. - The original Turkish analysis of this opium, 12.23 per cent morphine with 17.25 per cent moisture, corresponds to 14.78 per cent of morphine on the dry basis - a remarkably close check with the result now reported.

Opium samples

Loss in weight on drying (per cent)	Morphine content in per cent			
	as determined		on dry basis	
	I	II	I	II

Samples 40 to 46 were originally dried under reduced pressure and pulverized, and the resultant opium powders were analyzed. The loss in weight by the first drying is also given, and in parentheses, the morphine content corresponding to the original condition of the raw opium

40. Mongolia	1.92	8.37	8.42	8.53	8.58
	13.77	(7.22	7.26)		
41. Mongolia	2.82	11.91	11.97	12.26	12.32
	13.49	(10.30	10.36)		
42. Mongolia	3.49	11.91	11.94	12.34	12.37
	12.13	(10.47	10.49)		
43. China	2.61	10.41	10.12	10.69	10.39
	10.34	(9.33	9.07)		
44. China	1.06	9.16	9.09	9.26	9.19
	14.87	(7.80	7.74)		
45. China	1.74	10.14	9.92	10.32	10.10
	13.83	(8.74	8.55)		
46. China	0.65	7.52	7.28	7.57	7.33
	14.67	(6.42	6.21)		

Conclusions

23. The results obtained may be useful for comparisons with those yielded by other precipitation methods and extraction methods. In making such comparisons it should not be forgotten that the assay of the Japanese Pharmacopoeia (like that of the United States Pharmacopoeia but unlike the assays of the British Pharmacopoeia and the International Pharmacopoeia) does not add any correction.
24. The number of samples analyzed is too few for hard and fast conclusions regarding the bearing of the morphine assay on origin determinations. However, it is clear that there is some relation of morphine content to origin, as shown by the tabulation in the following paragraph.
25. Classification of samples in certain groups according to morphine content on the anhydrous basis, as determined by precipitation assay.

Total number of samples and origin	Number of samples				
	Below 9 per cent	Below 11 per cent	11 to 13 per cent	Above 13 per cent	Above 14 per cent
19 - Greece, Turkey, Yugoslavia	0	1	2	16	14
13 - India, Indochina, Iran, Japan	1	2	10	1	0
14 - China, Korea, Mongolia	6	9	5	0	0

26. It may also be noted that only one sample was found from any country with less than 7 per cent of morphine on the anhydrous basis, and that the results range up to more than 19 per cent of morphine.

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THE ASSAY, CHARACTERISTICS, COMPOSITION,
AND ORIGIN OF OPIUM*

No. 16

A Study of the United Nations Samples of Opium

By Dr. G. Panopoulos and Mr. A. Vassiliou,
General Chemical States Laboratory,
Athens, Greece

In continuation of the international programme of scientific research undertaken pursuant to resolutions 159 II C (VII) and 246 F (IX), adopted by the Economic and Social Council on 3 August 1948 and 6 July 1949 respectively, the Secretary-General is presenting the following paper contributed by Dr. Panopoulos and Mr. Vassiliou, two Greek scientists who are collaborating in the programme. The paper continues the study of opium characteristics which may make it possible to determine the origin of opium seized from the illicit traffic, and includes discussion of the authors' theory of the origin of differing opium characteristics and the limitations upon origin determinations.

The authors develop the test of general appearance, describing various qualities of the opium that may be noted, and recommending the use of a magnifying glass and ultra-violet light to supplement the usual examination. They have also studied the quantity and appearance of the ash, and quantitative determinations of the various forms of nitrogen in the opium. They conclude that the characteristics studied give good indications of origin, but they do not regard the problem as solved by the studies made.

* Inclusion of an article by contributing scientists in this series does not necessarily mean that the Secretariat shares the views expressed.

A STUDY OF THE UNITED NATIONS SAMPLES OF OPIUM

By Dr. G. Panopoulos and Mr. A. Vassiliou

Purpose of the paper

1. The purpose of this paper is to present a study of the United Nations samples of opium. This study was made with the view of discovering characteristics that may be useful in the determination of origin.
2. The study covers observations on the appearance, both by the unaided eye and through a magnifying glass, and also the appearance under ultra-violet light; the colour of the ash, both by natural light and under ultra-violet light; and quantitative determination of the ash and of the several forms of nitrogen in the opium.

Discussion

3. Defining the characteristics of opium which show its origin in a particular country is a difficult problem, as the physical properties and chemical constituents and the relations between them are dependent on many factors.
4. The method of cultivating the plant, the general weather conditions, how the opium is collected, the way the lumps of opium are packed and stored, are among the factors which determine the characteristics of the particular opium.
5. It may be possible that these conditions so affect the opium produced that even seeds from plants yielding a known type will produce a different type of opium and so it might be impossible to say that opium of a particular region has certain definite ratios between its constituents.
6. It is also to be noted that the distinction according to country is primarily political, though perhaps finally ethnological or geographical; but the factors causing the production of different types of opium are geological, climatic, and technical.
7. Thus it is natural that the plants cultivated in two different continents, but under the same climatic and geological conditions, should have the same percentage composition of the opium, if the technical conditions remain the same. Doubtless it would be rare, though not impossible, to find so many factors agreeing. Therefore, it is natural for the products to differ; but again such

differences vary between maxima and minima, and in different regions the variations will overlap, so that they cannot be completely separated according to countries or ethnological and geographical regions.

8. These results are noted in the products of one country if the technical conditions of cultivation, collection, and storage differ. So the products of Turkey not only present differences when they come from different districts but also products of the same district may sometimes show a different proportion in some of the relationships between constituents.

9. On this basis it is not possible to define the characteristics showing the origin of the opium by merely examining one sample from a district for the percentages of its constituents and the relations between them. It is a matter of examining numerous samples from a series of harvests. Then from the statistics we would draw conclusions by which we could probably distinguish the groups from different districts. Such distinctions could be made so probable that they would be but little short of certainty.

10. We wish to emphasize that the observation of characteristics of an annual harvest may make it possible to recognize opium from this harvest, but we consider that it would be unsafe to conclude that later harvests would necessarily have the same characteristics.

11. We have received the following samples of opium from the Opium Distribution Centre of the United Nations:

1 sample	originating from	China
3 samples	"	India
3 samples	"	Indochina
2 samples	"	Korea
15 samples	"	Turkey
<u>2 samples</u>	"	Yugoslavia

Total: 26 samples

12. In the tables below the characteristics of these samples are compared.

TABLE I

Serial No.	Details of the origin of samples	Colour	Appearance through a magnifying glass	Appearance of the fragment	Fragile or not	Remarks
TURKEY, Nos.1 to 15						
1	AYDIN No. 1 (C)	Dark brown	Many light brown spots	Vitreous	Almost unbreakable	Has residue of leaves; pressed it sticks to fingers.
2	AFYON No. 2 (B)	"	Light brown pockets with white spots	Granular	Fragile	" "
3	ÇORUM No. 3 (B)	Brownish red	Small metallic coloured spots as well as light brownish red pockets	Vitreous	Vitreous compact	" "
4	CAL No. 4 (B)	Dark brown	Granular with light brownish red spots and mould	Granular	Fragile	" "
5	USAK No. 5 (B)	Dark brownish red	Finely grained with lighter coloured spots and with white grooves	Finely grained	Fragile	" "
6	NALLIHAN No.6 (B)	Dark brown with lighter coloured grooves	Uneven surface with different coloured spots to semi-white	Granular dull surface	Fragile	" "
7	MERZIFON No.7 (C)	Dark brown	Small spots of a lighter colour	Granular with small pockets	Fragile	" "
8	BURDUR No. 8 (B)	"	Small spots of a lighter colour with many white grooves (mould)	Uneven with white leavings	Fragile	" "
9	KULA No.9 (B)	"	"	Uneven	Fragile with grain	" "
10	SANDIELI No.10 (B)	Dark brown	Many spots of a lighter colour with many white grooves (mould)	Uneven	Fragile with grain	It has residue of leaves; pressed does not stick to fingers
11	AKSEHIR No.11 (B)	"	Uneven surface with metallic shine in parts	Uneven	Fragile	" "

Serial No.	Details of the origin of samples	Colour	Appearance through a magnifying glass	Appearance of the fragment	Fragile or not	Remarks
12	ZILE No. 12 (C)	Dark brown	Granular spots of a lighter colour with empty apertures	Granular	Fragile	It has residues of leaves; pressed does not stick to the fingers
13	ISPARTA No.13 (B)	"	Spots of a lighter colour and white spots	Uneven granular	Fragile	" "
14	MAIATYA No.14 (C)	Brownish red	Spots of a lighter colour with red grooves	Glossy	Fragile	It has residues of leaves; pressed sticks to fingers
15	EXPORTATION No.15	Dark brown	Spots of a lighter colour with white lines (mould)	Granular	Fragile	It has residue of leaves; pressed does not stick to the fingers
16	INDIA, BANARAS No.1	Black	Waxy surface	Well-shaped like wax	Fragile like putty	Pressed sticks to the fingers; greasy in touch
17	INDIA, MAIWA No.2	Brownish black	Smooth, even surface	Even, dull surface like Spanish wax	Fragile tough in all directions	It has residues of leaves; sticks lightly to the fingers; greasy to touch
18	INDIA, EXCISE No.3	Dark brownish black	" " " "	" "	"	Greasy to touch
19	KOREA No. 1	Brownish black	Brownish black, even, dull surface	Vitreous	Fragile	No residue of leaves; pressed does not stick to the fingers
20	KOREA No. 2	Brownish black	Spots of a lighter brown colour; even surface	Like a fragment of Spanish wax	Tough	Greasy to touch
21	YUGOSLAVIA No.1	Dark brown	Smooth surface with spots of a lighter colour	Even (dull) surface like Spanish wax	Fragile	It has residues of leaves; pressed does not stick

Serial No.	Details of the origin of samples	Colour	Appearance through a magnifying glass	Appearance of the fragment	Fragile or not	Remarks
22	YUGOSLAVIA No.2	Dark brown and lighter in parts	Dark brown; lighter in parts	Uneven, granular	Fragile	It has residues of leaves; pressed does not stick
23	INDOCHINA LUANG-PHABANG (A)	Brownish red	Granular with spots of a lighter colour to brownish red	Granular	Very fragile and friable	Has pieces of white wrapping paper
24	INDOCHINA XIENG-KHOUANG (B)	Dark brownish black	Very glossy surface with spots of a lighter colour as well as apertures of different sizes	Dull surface like Spanish wax		Has residues of leaves; pressed sticks lightly
25	INDOCHINA LACKAY	"	"	"	"	"
26	CHINA (B)	Dark brown	Smooth and even surface	"	Fragile	It has not residue of leaves; pressed does not stick
27	GREECE THRAKI (1)	Dark brown	Uneven surface and of a lighter colour in parts	Uneven granular	Fragile	It has residues of leaves; pressed does not stick to fingers
28	GREECE THRAKI (2)	"	"	"	"	"

13. From the data in Table I, it is concluded that the samples of opium show considerable characteristic distinction in groups. Thus the group of samples from India show, besides the brownish black colour, a very glossy surface, and a broken fragment has an even surface, lusterless like Spanish wax, without little pockets, and greasy to the touch.
14. The samples from Korea, Indochina, and China have a somewhat similar appearance, in distinction from all the more numerous samples from Turkey, Yugoslavia and Greece.
15. The samples from Yugoslavia and Greece are alike in appearance.
16. In Table II we give the appearance of the ash of the samples, under natural light and also as seen by the reflection of ultra-violet light. The colour of the ash in the two cases may indicate some characteristic related to origin.
17. The colour of the ash of sample 26 (China B), which under natural light is in various shades from light pink to brick colour, seems to be characteristic for this type of opium.
18. One of the means of distinguishing types of opium was thought to be the relationship between the total water-soluble nitrogen and the amount of nitrogen in the morphine.
19. For determination of the total water-soluble nitrogen the following method was used:

Four grammes of well-ground opium, dried at 60-70° C, are taken, and put in a mortar with 4 cc water and rubbed to a homogeneous mixture. After rubbing for 10 minutes the mixture is rinsed into a pyrex beaker with 40 cc water. After standing for an hour, with frequent stirring with a glass rod, the solution is filtered into a volumetric vessel of 100 cc. The glass and the filter paper are washed and the volume made up to 100 cc with distilled water. 10 cc of this solution is put in a Kjeldahl flask, with 25 cc of concentrated sulfuric acid, 10 grammes of potassium sulfate, and 1 gramme of copper sulfate. This is left for half an hour. It is then heated for half an hour by a small flame, and afterward the nitrogen is determined in the usual way.
20. The following process was used to determine volatile or ammoniacal nitrogen (all the volatile basic compounds determined by this process are calculated as ammoniacal nitrogen):

Table II

Serial No.	No. of origin of samples	Appearance of Ash	
		Natural light	Reflection of Ultra-Violet
1.	TURKEY, Nos 1 to 15	Greyish colour	Brownish red; violet in parts
2.	AYDIN No. 1 (C)	Greyish colour;	Brown; violet in parts
	AFYON No. 2 (B)	yellowish in parts	
3.	ÇORUM No. 3 (B)	Whitish; light pink in parts	Brownish black
4.	ÇAL No. 4 (B)	Greyish colour;	Light brownish red, with some spots bright orange-reddish
		yellowish in parts	
5.	USAK No. 5 (B)	Greyish	Brownish black
6.	NALLIHAN No.6 (B)	Semi-white; greyish in parts	Brown; violet in parts
7.	MERZIFON No.7 (C)	White	Violet
8.	BURDUR No. 8 (B)	Semi-white; yellowish in parts	Light brownish red; violet in parts
9.	KULA No. 9 (B)	Semi-white; yellowish in parts	Greyish; with some bright orange-red spots
10.	SANDIQLI No.10 (B)	Greyish; in parts yellowish	Brownish black
11.	AKSEHIR No.11 (B)	Orange-yellowish	Orange-yellowish
12.	ZILE No. 12 (C)	White	Violet with many bright orange-reddish spots
13.	ISPARTA No.13 (B)	Semi-white; light grey in parts	Brownish black
14.	MALATYA No.14 (C)	White	Violet with many white spots of reddish orange colour
15.	EXPORTATION No.15	Grey colour; orange-reddish in parts	Brownish black; grey in parts
16.	INDIA, BANARES	Orange (light)	Brownish black
17.	INDIA, MALWA	White	Violet with some bright orange-reddish spots and a few light blue
18.	INDIA, EXCISE	White	Dark violet with some bright orange-reddish spots
19.	KOREA No. 1	Light grey; pinkish red in parts	Dark violet
20.	KOREA No. 2	Grey	Dark violet
21.	YUGOSLAVIA No. 1	White	Dark violet
22.	YUGOSLAVIA No. 2	Light pink; white in parts	Dark violet
23.	INDOCHINA LUANG PRABANG (A)	White	Dark violet
24.	INDOCHINA XIENG KHOUANG	White	Dark violet with a few bright orange-reddish spots
25.	INDOCHINA LAOKAY	Very light yellowish-red	" " "
26.	CHINA (B)	Light pinkish brick	Dark violet
27.	GREECE, THESSALY No. 1	Light greyish	Dark violet
28.	THESSALY No. 2	Light greyish	Dark violet

50 cc of the solution of water-soluble compounds is put in an apparatus for distilling off ammonia (in the usual determination of nitrogen), and 5 cc of a 30% solution of NaOH is added. The distillate is caught in a flask containing a measured volume of a normal solution of acid. After the distillation the excess of acid is titrated, with methyl orange as indicator.

21. The difference in percentage between the total water-soluble nitrogen and the ammoniacal nitrogen is the non-volatile water-soluble nitrogen.
22. The relationship of the non-volatile nitrogen to the nitrogen of the morphine content gives some characteristic indications of origin.
23. The results of the nitrogen determinations mentioned are given in Table III. Also the percentage of ash for the samples dried at 60-70° C, and its relationship to the non-volatile water-soluble nitrogen, are included in this Table.
24. Two charts are attached, which show graphically the relationship between the nitrogen of the morphine and the total non-volatile water-soluble nitrogen. Chart 1 shows the relationship for all the samples separately and Chart 2 shows it for the average of the samples from each country.
25. From Chart 1 it is evident that these relationships might be used as an indication of origin. For example, Nos. 19 and 20, the samples from Korea, are easily distinguished from the others. No. 26 from China, and Nos. 23 and 24 from Indochina, can also be distinguished.
26. Photographs of characteristic broken surfaces of some of the samples are also attached. In these photographs the uneven and granular surface of the Turkish samples may be contrasted with the smooth dull surface of the samples from India, Indochina, and China. Coloured photographs would of course show the characteristic differences much more clearly than is possible in black and white.

Conclusions

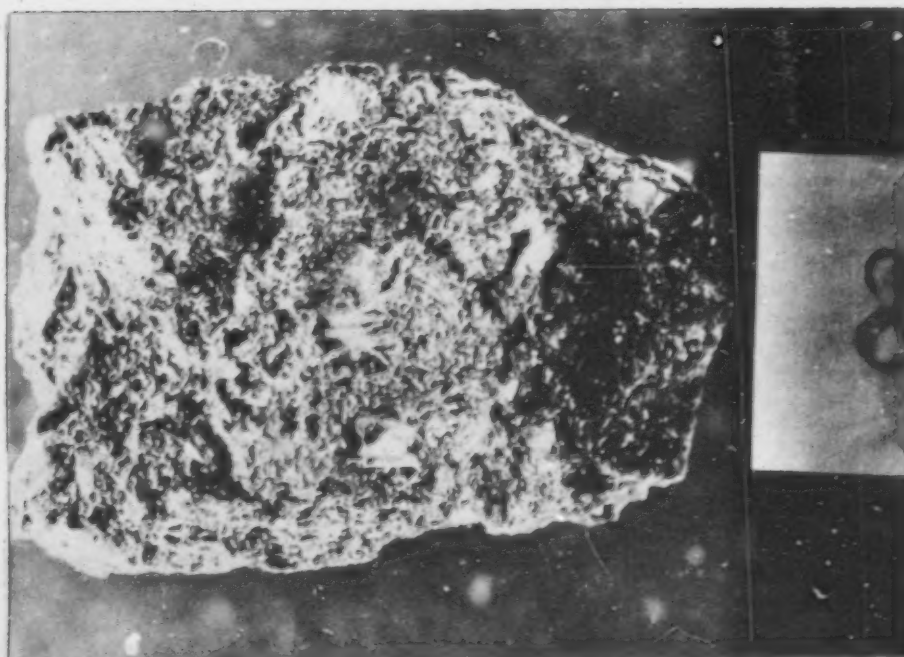
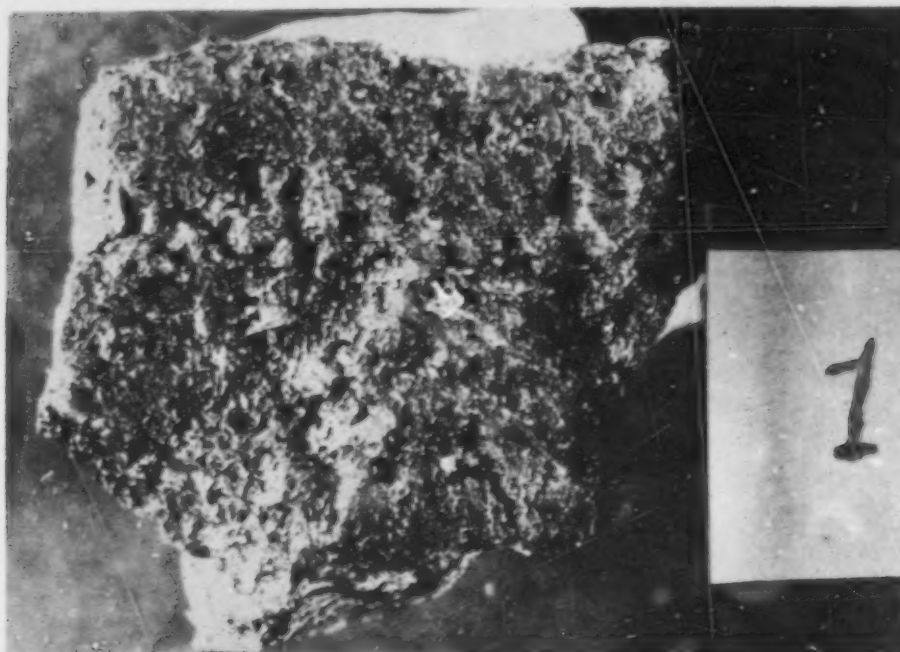
27. A detailed examination of the sample, including observation under a hand lens and under ultra-violet, will reveal good indications of its origin.
28. The amount of ash, its colour under natural light and under ultra-violet, will add new characteristic elements which are important in some cases.

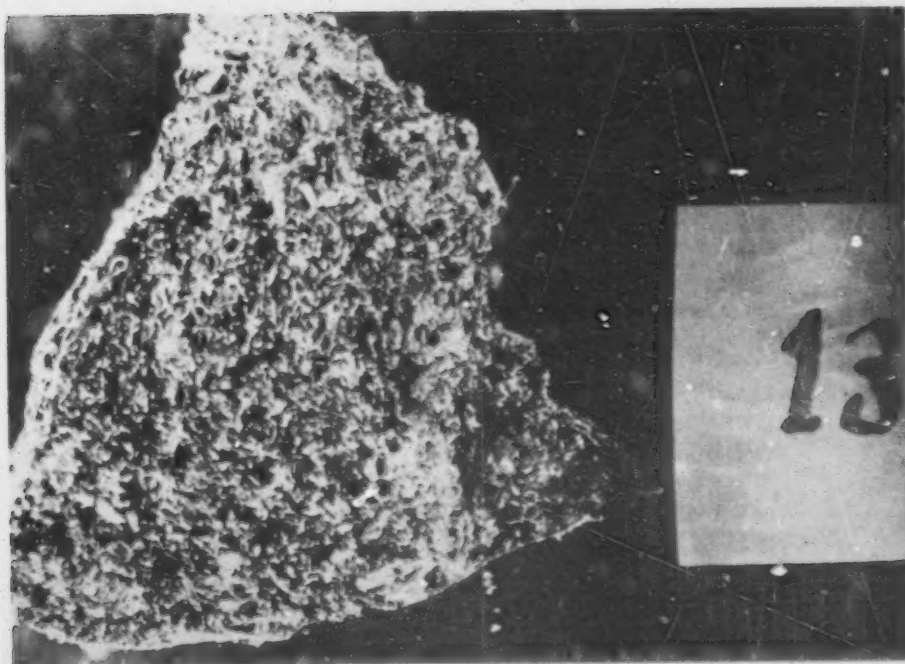
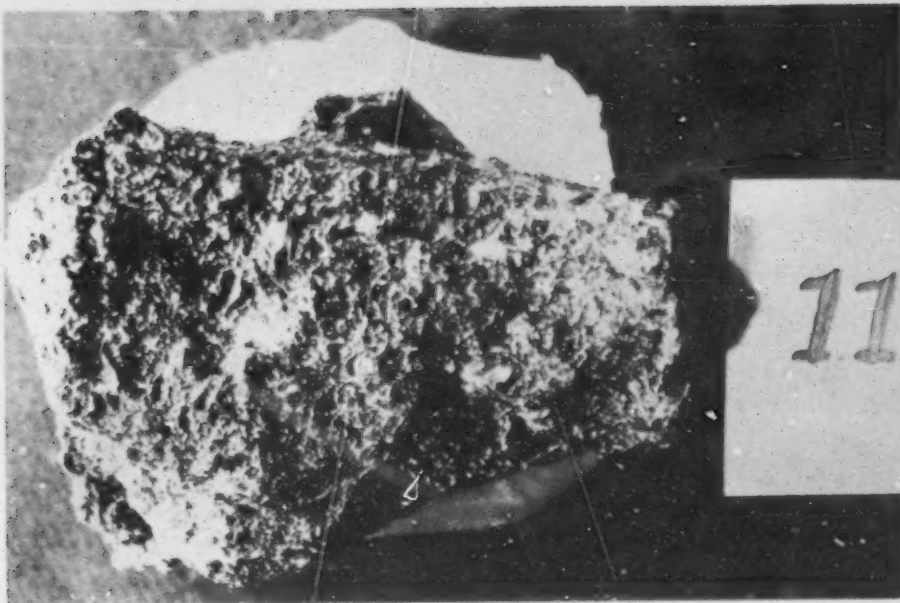
TABLE III

Serial No.	Country	Ash	Nitrogen of morphine	Total water-soluble nitrogen	Ammoniacal nitrogen	Non-volatile water-soluble nitrogen	Relationship of non-volatile water-soluble nitrogen to the nitrogen of morphine	Relationship of non-volatile water-soluble nitrogen to the amount of ash
1.	TURKEY	3.28	0.716	2.5025	0.273	2.2295	3.110	1.47
2.	"	5.81	0.687	1.82	0.210	1.610	2.343	3.60
3.	"	3.00	0.909	2.66	0.293	2.387	2.626	1.25
4.	"	3.71	0.7385	2.205	0.182	2.023	2.737	1.35
5.	"	3.51	0.800	1.680	0.189	1.491	1.864	2.35
6.	"	5.37	0.597	1.855	0.245	1.610	2.690	3.33
7.	"	2.37	0.870	2.100	0.210	1.890	2.171	1.25
8.	"	4.57	0.6855	1.645	0.196	1.449	2.110	3.14
9.	"	4.37	0.795	1.995	0.217	1.778	2.236	2.46
10.	"	5.34	0.613	1.750	0.196	1.554	2.535	3.43
11.	"	5.88	0.550	1.715	0.196	1.519	2.762	3.86
12.	"	3.72	0.4981	2.415	0.231	2.184	4.390	1.70
13.	"	4.61	0.5074	1.925	0.203	1.722	3.390	2.67
14.	"	3.52	0.5963	2.275	0.245	2.030	3.440	1.73
15.	"	6.84	0.6605	1.96	0.203	1.757	2.660	3.88
16.	INDIA	3.88	0.4901	2.485	0.308	2.177	4.44	1.78
17.	"	3.50	0.515	2.310	0.322	1.980	3.92	1.77
18.	"	3.63	0.426	2.625	0.238	2.387	5.60	1.52
19.	KOREA	6.87	0.247	6.100	1.370	4.730	19.17	1.45
20.	"	12.40	0.2267	4.620	0.945	3.675	16.21	3.37
21.	YUGOSLAVIA	3.95	0.795	2.520	0.231	2.289	2.88	1.72
22.	"	4.15	0.736	2.030	0.266	1.764	2.40	2.35
23.	"	2.13	0.643	2.590	0.273	2.317	3.60	0.91
24.	INDOCHINA	2.12	0.520	2.765	0.378	2.387	4.600	0.92
25.	"	3.29	0.7017	2.345	0.283	2.062	2.935	1.58
26.	CHINA	9.82	0.3974	2.625	0.807	1.818	4.550	5.40
27.	"	2.690	0.8833	2.700	0.259	2.441	2.763	1.10
28.	GREECE	3.070	0.7935	2.654	0.247	2.407	3.033	1.27

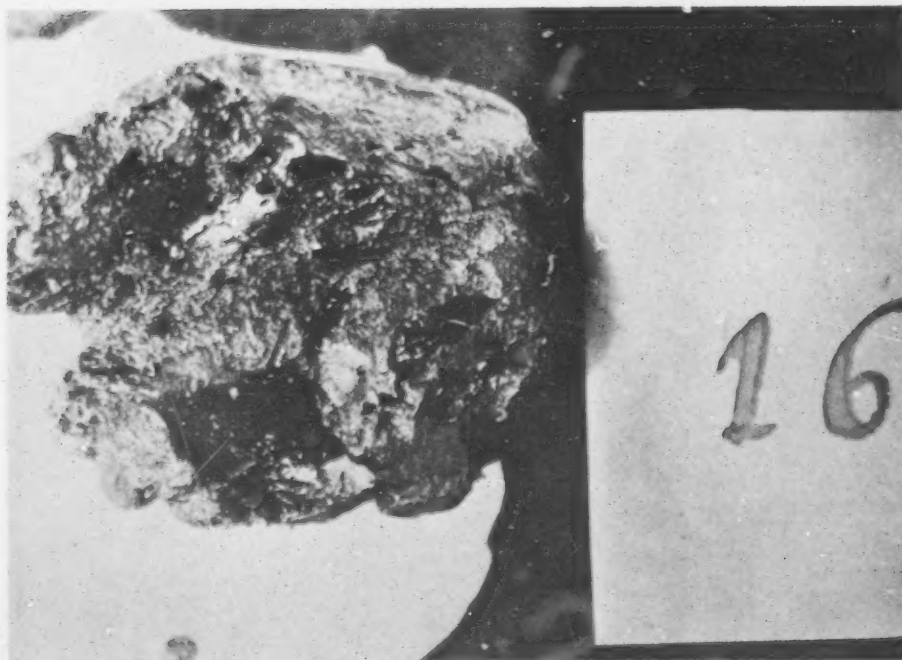
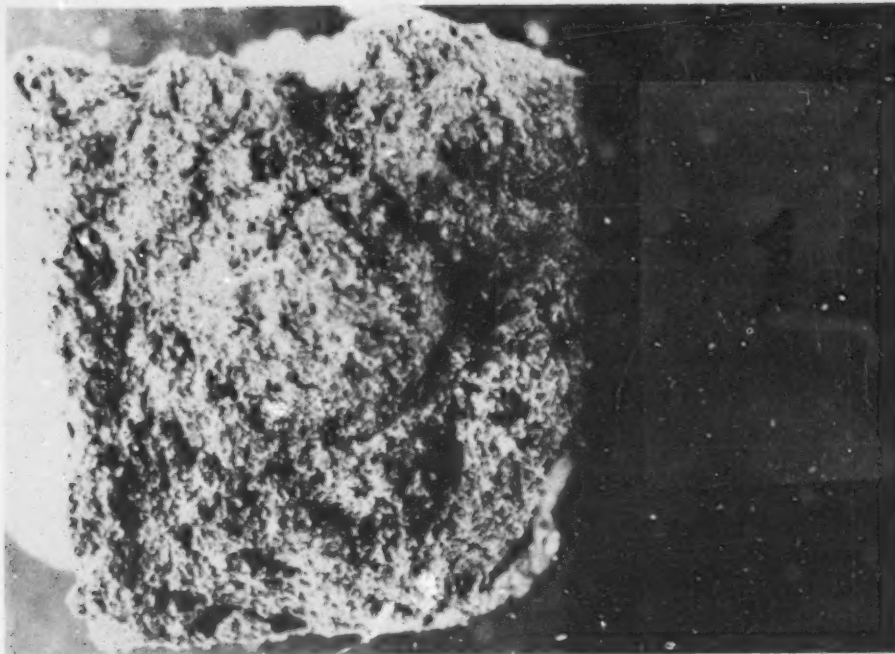
29. The amount of morphine or of its nitrogen, and the comparison of that quantity to the total non-volatile nitrogen, add new elements which characterize the opium.
30. With all the things mentioned we do not consider the problem of determining the origin of opium as solved, but that other elements added to those described will combine to yield the safest possible determinations.
31. Research on other aspects of the same problem will be continued by us, and also studies in the determination of morphine in opium.

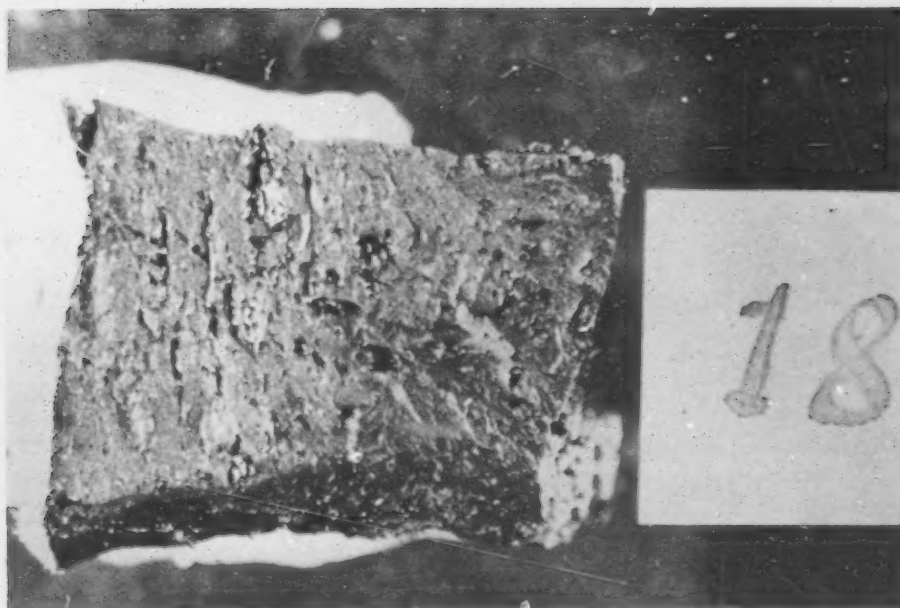
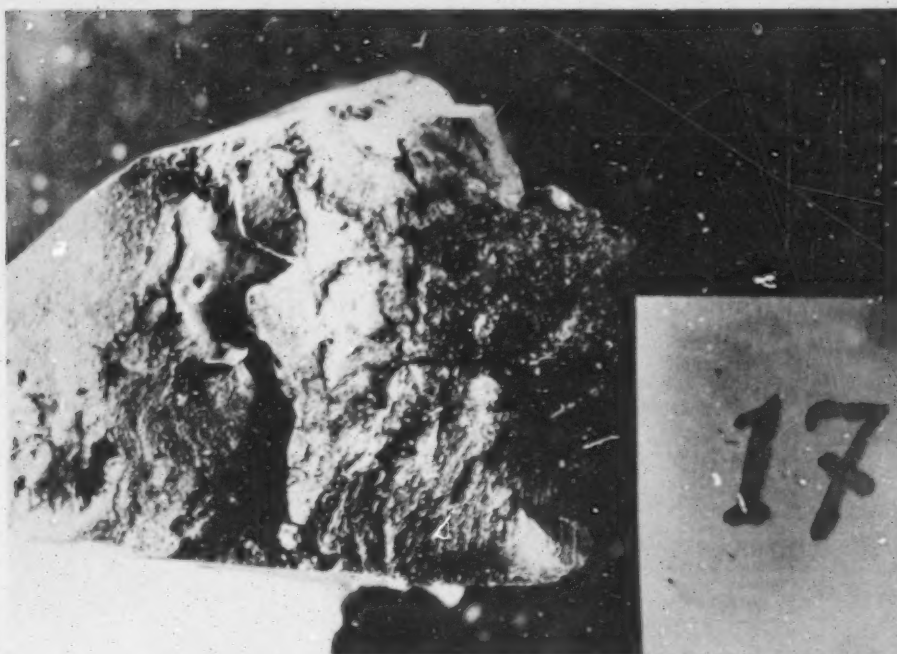
PHOTOGRAPHS OF FRAGMENTS OF OPIUM SAMPLES (identified by number)
showing the characteristic differences in the broken surfaces.





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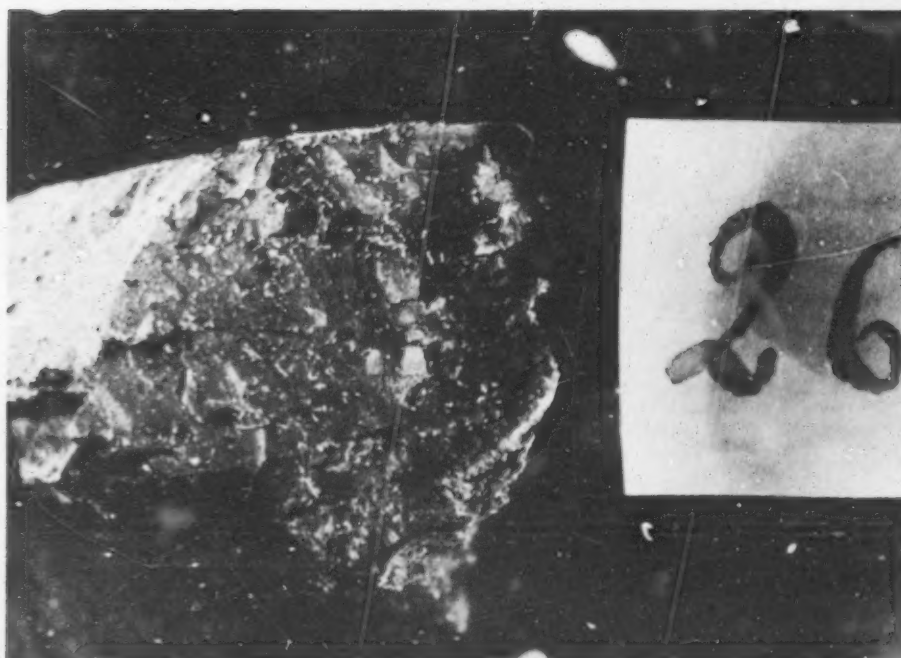
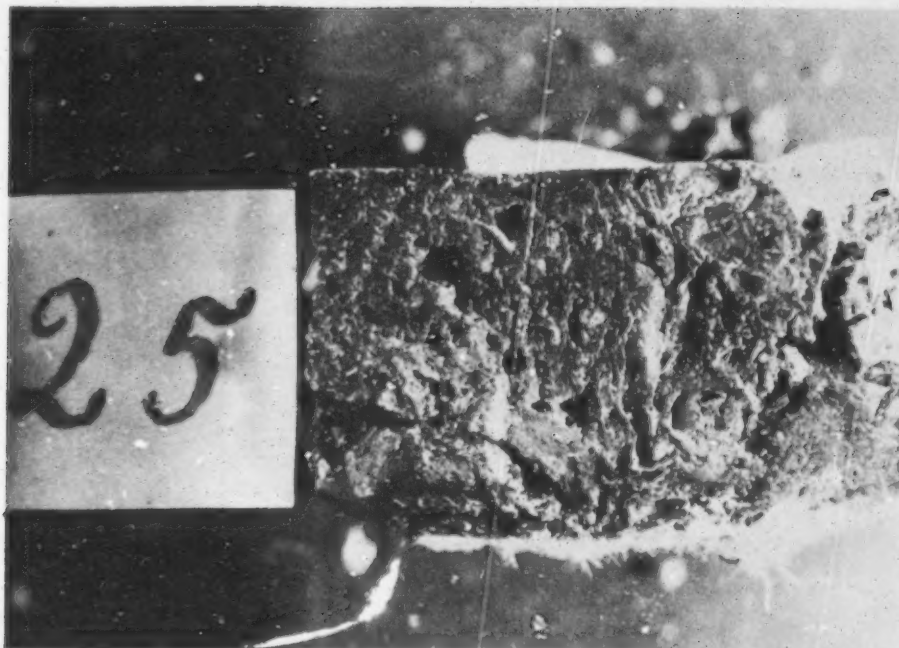


CHART 1. Relation of the nitrogen of the morphine to the total non-volatile water-soluble nitrogen for each of the opium samples examined.

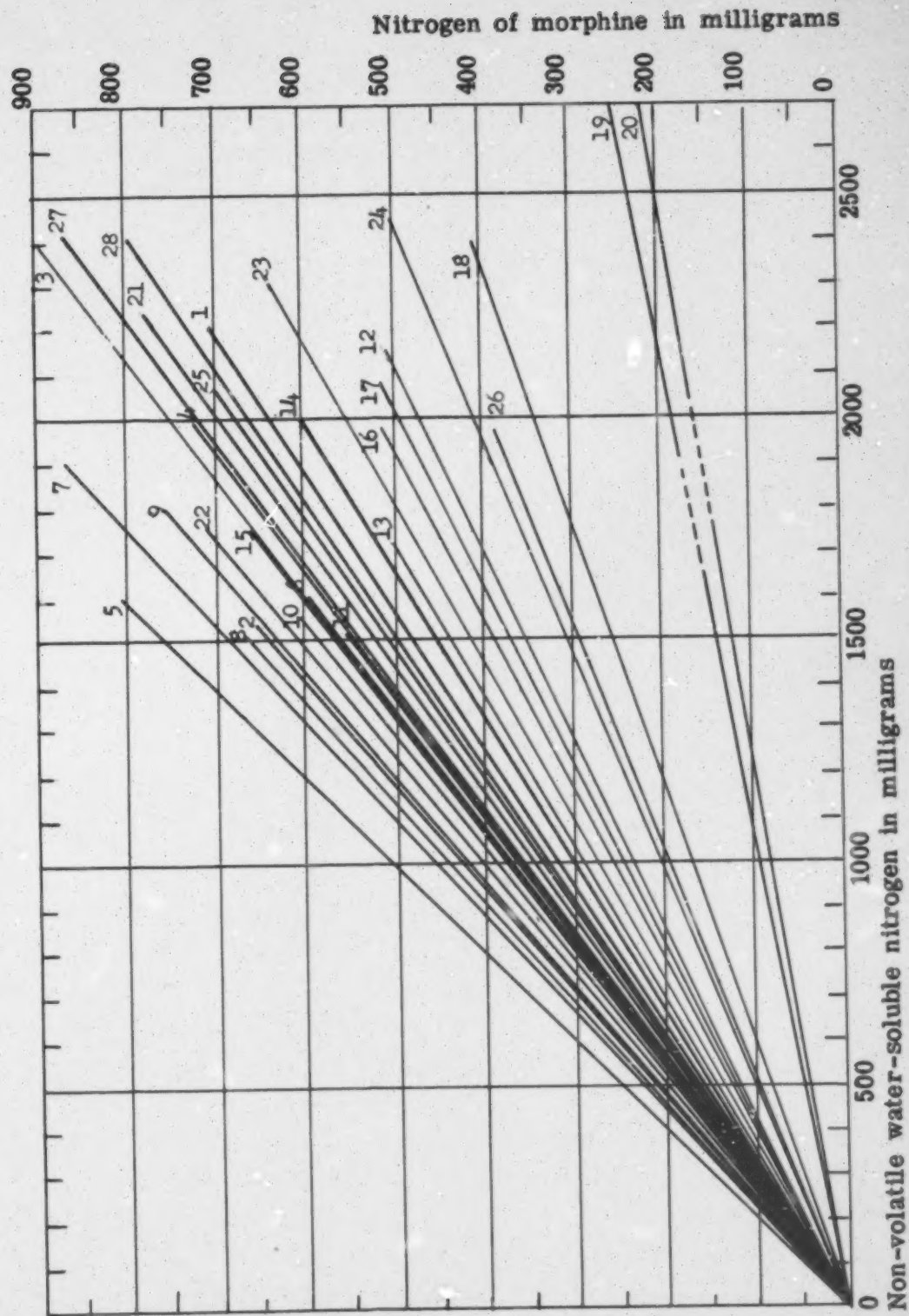
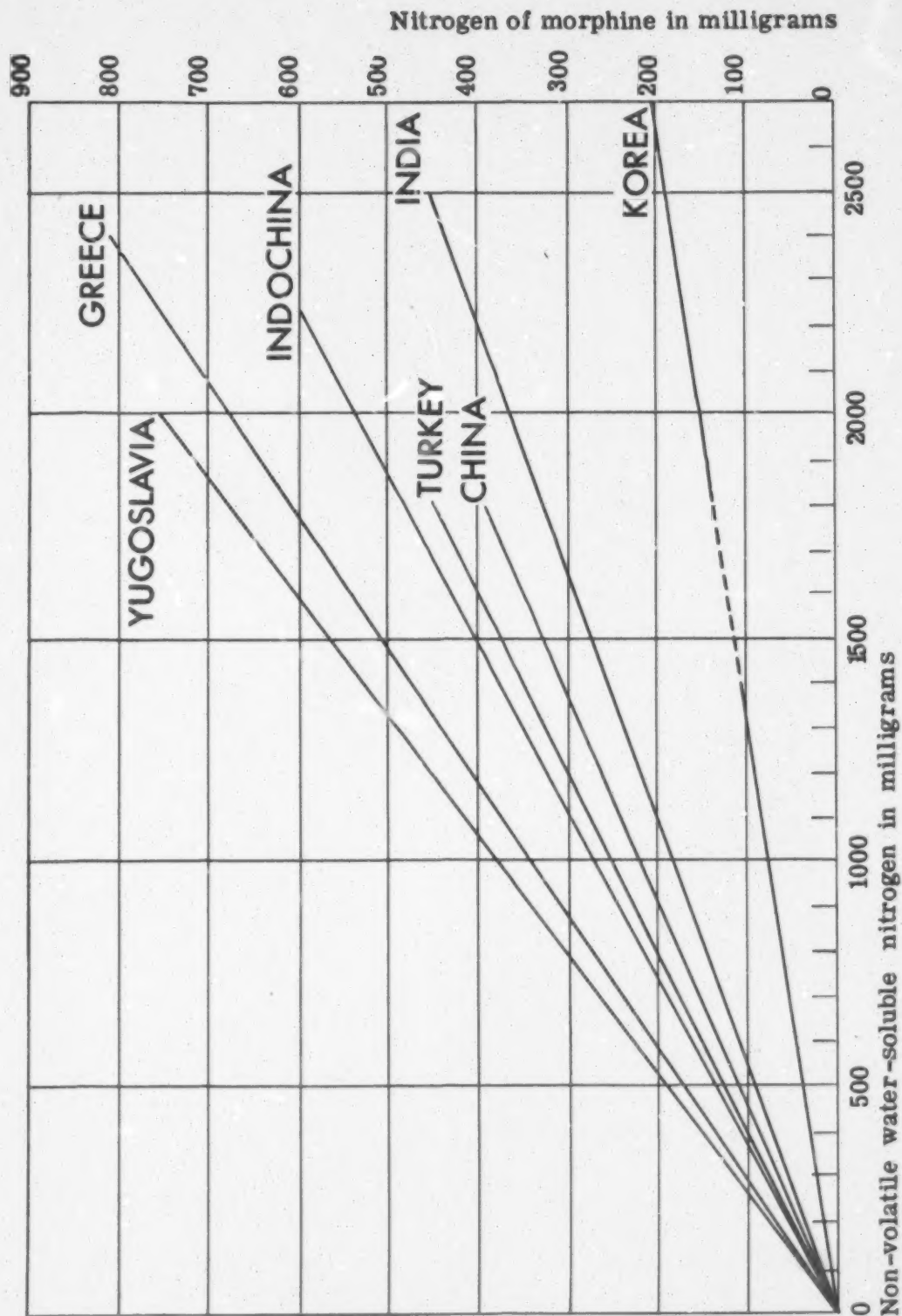


CHART 2. Average relation, for the samples from each country separately, of the nitrogen of the morphine to the total non-volatile water-soluble nitrogen of the opium.



UNITED NATIONS

SECRETARIAT



GENERAL

ST/SGA/SER.K/17
25 March 1953

ORIGINAL: ENGLISH

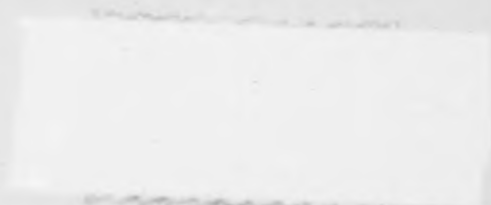
THE ASSAY, CHARACTERISTICS, COMPOSITION,
AND ORIGIN OF OPIUM

No. 17

Extractive Determination of Thebaine and Papaverine in Opium

In continuation of the international programme of scientific research on opium undertaken pursuant to resolutions 159 II C (VII) and 246 F (IX), adopted by the Economic and Social Council on 3 August 1948 and 6 July 1949 respectively, the Secretary-General is presenting the following paper prepared by the chemists of the United Nations Secretariat. These new methods for thebaine and papaverine are expected to be useful in the commercial evaluation of different types of opium, in control over the production of opium alkaloids, and in the problem of determining the origin of illicit opium by scientific means.

53-07871

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Charts 1, 2, and 3 following paragraph 34

Table I following paragraph 84

EXTRACTIVE DETERMINATION OF THEBAINE AND PAPAVERINE IN OPIUM

Introduction

1. The five principal alkaloids of opium, in amount, are morphine, narcotine, codeine, thebaine, and papaverine. The four economic alkaloids of opium are the same with the exception of narcotine, which has very little known value as yet.
2. Methods have been previously given in this series for the determination of morphine, narcotine, and codeine in opium. The determination of thebaine and papaverine therefore completes the determination of the five alkaloids present in the largest amounts, and the assay of opium for the four economic alkaloids.
3. Thebaine is a phenanthrene alkaloid of opium -- having the same fundamental structure as morphine and codeine -- and is under international narcotics control. Papaverine, however, -- like narcotine -- does not have the phenanthrene structure and is not under international control; but it is sometimes nationally controlled.
4. The percentages of thebaine and papaverine vary extremely in different types of opium. It is therefore expected that the determinations will be useful in solving the enforcement problem of the origin of illicit opium, although the study of known samples is still quite incomplete.
5. The four economic alkaloids are also the four principal alkaloids in smoking opium, since most of the narcotine is discarded in the preparation of opium for smoking purposes. The best chance of determining the origin of the original opium by chemical means, when it has been made into smoking opium, therefore seems to rest with determinations of the relative amounts, or ratios, of these four alkaloids, thebaine and papaverine as well as morphine and codeine.
6. The new methods will therefore be useful in the commercial evaluation of different types of opium; in analytical "control" of the factory extraction of opium alkaloids; possibly in the control and accounting of narcotic drugs by national and international authorities; and in the problem of determining the origin of illicit opium by scientific means.

Discussion

7. Of the five major alkaloids of opium, narcotine and papaverine are, in general, the easiest to extract with organic solvents, morphine and codeine the most difficult,

and thebaine in between. Morphine at one extreme can be separated from the other major alkaloids by means of its solubility in alkali. So can narcotine at the other extreme, by causing its lactone ring to open. Papaverine, thebaine, and codeine, however, are all non-phenolic, non-acidic alkaloids of generally similar properties in regard to extractions, differing in degree rather than kind in such properties.

8. Since thebaine is between papaverine and codeine in extractive properties, two distinct and difficult separations are involved in its determination.
9. Papaverine also has extractive properties between two other alkaloids, thebaine on the one side and narcotine on the other. It most resembles narcotine; but the characteristics of narcotine are changed by causing its lactone ring to open, and its separation from papaverine can then be rather easily effected. Therefore the separation of papaverine from thebaine is the more difficult to make complete, and accomplishing it makes it possible and even convenient to determine both thebaine and papaverine on the same sample of opium.
10. The first problem is to get the alkaloids out of the solid opium.
11. Thebaine and the alkaloids more easily extracted (narcotine and papaverine of the major alkaloids) can be taken out of dried, powdered opium by chloroform, using a Soxhlet extractor. In fact, tentative methods along this line were offered in E/CN.7/117/Add.2, 22 September 1948. However, it has not been proved that this extraction is always complete for thebaine.
12. Another method is to extract the alkaloids from the solid opium by means of acidified water. This has the advantage that it is not necessary first to dry and powder the opium. It is also the most general method for getting the alkaloids into solution, so that by varying the later procedure, any of the other alkaloids of opium might be determined on the same extract.
13. An extraction of all the alkaloids is best accomplished with rather strong acetic acid. The mineral acids (hydrochloric and sulfuric have been generally used) change the "porphyroxine-maconidine" into a red compound; and if such acids are diluted they do not readily dissolve the narcotine crystals; and they do not disintegrate the opium itself nearly as readily as acetic acid does. As no narcotine determination is included in the present method, a strength of 5 per cent acetic acid is sufficient for the aqueous extraction.

14. The next problem is, what specific method, in particular what solvent, is to be used for separating the thebaine from the codeine, or from the papaverine?

15. Several combinations of acids and solvents can be used. From unbuffered acetic acid solution carbon tetrachloride will gradually take out narcotine and papaverine but not thebaine; if the solution is appropriately buffered with sodium acetate, thebaine can also be shaken out while the codeine is retained. Chloroform, a more effective solvent, will extract thebaine, as well as narcotine and papaverine, but not codeine, from unbuffered acetic acid. Dilute sulfuric acid will retain thebaine fairly well even against chloroform, while permitting the extraction of narcotine and papaverine.

16. Carbon tetrachloride has much more tendency than chloroform to form emulsions, and if it is to be used for the major separations of the non-phenolic alkaloids, a preliminary solvent extraction of the aqueous extract of opium is necessary; e.g., by continuous extraction with ether from an alkaline medium. Chloroform, on the other hand, can be used for direct extraction of the original opium solution, and the same chloroformic solution can then be used for the major separations.

17. Another principle to be noted is the following: In separating an alkaloid from the solution of opium extractives, simply carrying it along in the original solvent, past various types of aqueous washing and cleaning solutions, is not sufficient for purification; -- the alkaloid should at some time be back-extracted into aqueous solution, in order to free it from non-alkaloidal substances which may be carried along in the organic solvent. After the back-extraction of thebaine from chloroform, the solvent can then be changed to carbon tetrachloride, which will not extract so much extraneous material as chloroform. A back-extraction of papaverine is also necessary, and here also the final extraction is made with carbon tetrachloride.

18. In working out the method, a difficulty was encountered in testing for completion of the thebaine extractions: some other substance is present which gives nearly the same colour reaction with (2 / 1) H_2SO_4 , the reagent previously used in testing for the elimination of thebaine in the codeine determination (E/CN.7/202, 18 September 1950). The other substance seems not to be extracted to any extent from opium by lime water, but there is quite a quantity of it in the acetic-acid extract. Thebaine gives almost as strong a colour with syrupy H_3PO_4 as with (2 / 1) H_2SO_4 , and a fairly strong orange even with concentrated HCl;

the other substance yields a very weak reaction with H_3PO_4 , and almost nothing with HCl. The presence of thebaine can therefore be recognized by these latter reagents.

19. Finally, the question of how much opium to use, must be answered. Having regard to the usual scale of laboratory procedures, the more opium that can be used conveniently, the better. At the same time the amounts of the alkaloids involved should be small enough not to require excessive quantities of solvents. The thebaine in 3 grammes opium is generally enough for a convenient titration with n/50 acid, and this amount of opium was selected for use in the method.

Principles of the Thebaine separation

20. The solid opium is extracted with dilute acetic acid, and the filtered solution is slightly buffered with sodium acetate, which tends to convert alkaloidal meconates into acetates. The solution is extracted with chloroform. The chloroform soon takes out all the narcotine, papaverine, and thebaine, along with some amounts of the minor alkaloids, including most of the cryptopine and protopine. A considerable part or even most of the codeine is also extracted.

21. The chloroform extracts are shaken first with three solutions of unbuffered acetic acid. These acid solutions take out the codeine, any traces of morphine that may have been extracted from the original opium solution, part of the cryptopine and other minor alkaloids, and a considerable amount of other impurities, including a considerable part of the one which yields a thebaine-like reaction with sulfuric acid. The completion of thebaine extraction is tested for with concentrated HCl.

22. The chloroform extracts are then washed with two alkali solutions. These remove a considerable amount of colored impurities, and the acidic alkaloid narceine. Any "alkaloid 3" (a minor phenolic alkaloid) that may have been carried this far, is likewise removed, but other minor phenolic alkaloids that are or may be present, are removed only partially, if at all, at this stage. The alkali solutions also have the necessary function of taking out acetate from the chloroform so that in the next stage the remaining alkaloids may be separated by means of chloroform and dilute sulfuric acid.

23. The chloroform-extracts are then shaken with three solutions of dilute sulfuric acid, and this is the important stage at which thebaine is separated from the narcotine and papaverine. Fresh chloroform is used as long as necessary to

shake out all the narcotine and papaverine from the acid solutions. Any cryptopine, protopine, and "porphyroxine-meconidine" still in the chloroform at the beginning of this stage are left in the dilute acid solutions with the thebaine. The chloroform extracts are then shaken with dilute bicarbonate solution to eliminate any acidity, and reserved for the papaverine determination (paragraph 29).

24. The three solutions containing the thebaine in dilute sulfuric acid are washed with carbon tetrachloride, preparatory to a change of solvent, and combined. They are then made strongly alkaline with sodium hydroxide, and shaken out with carbon tetrachloride past an alkali wash. This treatment removes the greater part of the minor phenolic alkaloids still present, as well as other impurities that are carried past alkali by chloroform but not by carbon tetrachloride.

25. The CCl_4 extracts are then shaken with two solutions of dilute acetic acid buffered with sodium acetate. These aqueous solutions take out any codeine that may have got so far, most of the remaining cryptopine and part of the protopine, and most of the remaining impurity with the thebaine-like colour-reaction with sulfuric acid. The extracts are then shaken with dilute ammonia, washed with water, and run into a weighed dish, and the solvent evaporated off.

26. Generally, the separated thebaine crystallizes very readily beginning to do so on the sides of the dish even before the solvent has evaporated completely. In a few cases, which seem to be most often samples of Turkish or Yugoslav origin, crystallization-hindering impurities are still present. The samples of highest thebaine percentages (from Indochina and Iran) have yielded completely crystalline thebaine residues.

27. In all cases the residues have been titrated. The titration result is always somewhat lower than the weight result, more so when the thebaine refuses to crystallize than when it crystallizes completely, as it usually does.

28. If desired, the thebaine can be destroyed by boiling with strong hydrochloric acid (forming the phenolic thebenin) and the unchanged non-phenolic alkaloids extracted from alkaline solution. Only a little residue with a small titration, equivalent to less than a tenth of a per cent of the opium weight, has been found in the thebaine, in the tests that have been made in this way since the method was put in its present form. No correction has been applied to these thebaine values because it is thought that the losses of thebaine amount to at least as much. The other alkaloid thus found in the thebaine seems to be mainly protopine rather than

cryptopine. The latter is slightly closer to codeine in properties, than is protopine.

Principles of the Papaverine separation

29. The extraction from opium and the separation of papaverine and narcotine from the other opium alkaloids, and in particular from thebaine, have already been discussed (paragraphs 20 to 25). Chloroform is recovered from the solution containing the narcotine and papaverine by distillation to a comparatively small volume, and then the solution is poured into a dish and the remaining chloroform evaporated off.

30. The residue of alkaloids then heated in alcohol with potassium hydroxide. This opens the lactone-ring of narcotine and makes it alkali-soluble in water, and insoluble in carbon tetrachloride. The alcoholic solution is poured into water and the papaverine extracted with carbon tetrachloride.

31. It will be noted, however, that up to this point the papaverine has been extracted by the organic solvent in every case. Therefore, it is now back-extracted from the carbon tetrachloride by means of dilute sulfuric acid, and the carbon tetrachloride is reserved for recovery by distillation.

32. The acid solutions are then combined, made alkaline, and extracted with fresh carbon tetrachloride, past a small solution of unbuffered dilute acetic acid. This acid solution is precautionary and intended to hold back any thebaine that might have got into the narcotine-papaverine residue. The carbon tetrachloride extracts are then shaken with dilute ammonia and washed with water and filtered into a weighed dish. Fresh carbon tetrachloride is used as necessary to extract any papaverine held by the unbuffered acetic acid solution.

33. Thus far, the papaverine has been determined by weighing only, not by titration. However, the residue has been completely crystalline in all cases when any considerable amount has been present. The residues have been somewhat yellowish, possibly due to a little papaveraldine (Xanthaline). In a very few cases (two for Indochina and one for Afghanistan) the residue was extremely small and refused to crystallize, and qualitative test indicated that little if any actual papaverine was present. In other cases of low results crystalline papaverine was obtained.

Charts Summarizing the Method

34. Charts 1, 2, and 3 summarize the principles of the thebaine and papaverine separations.

CHART 1

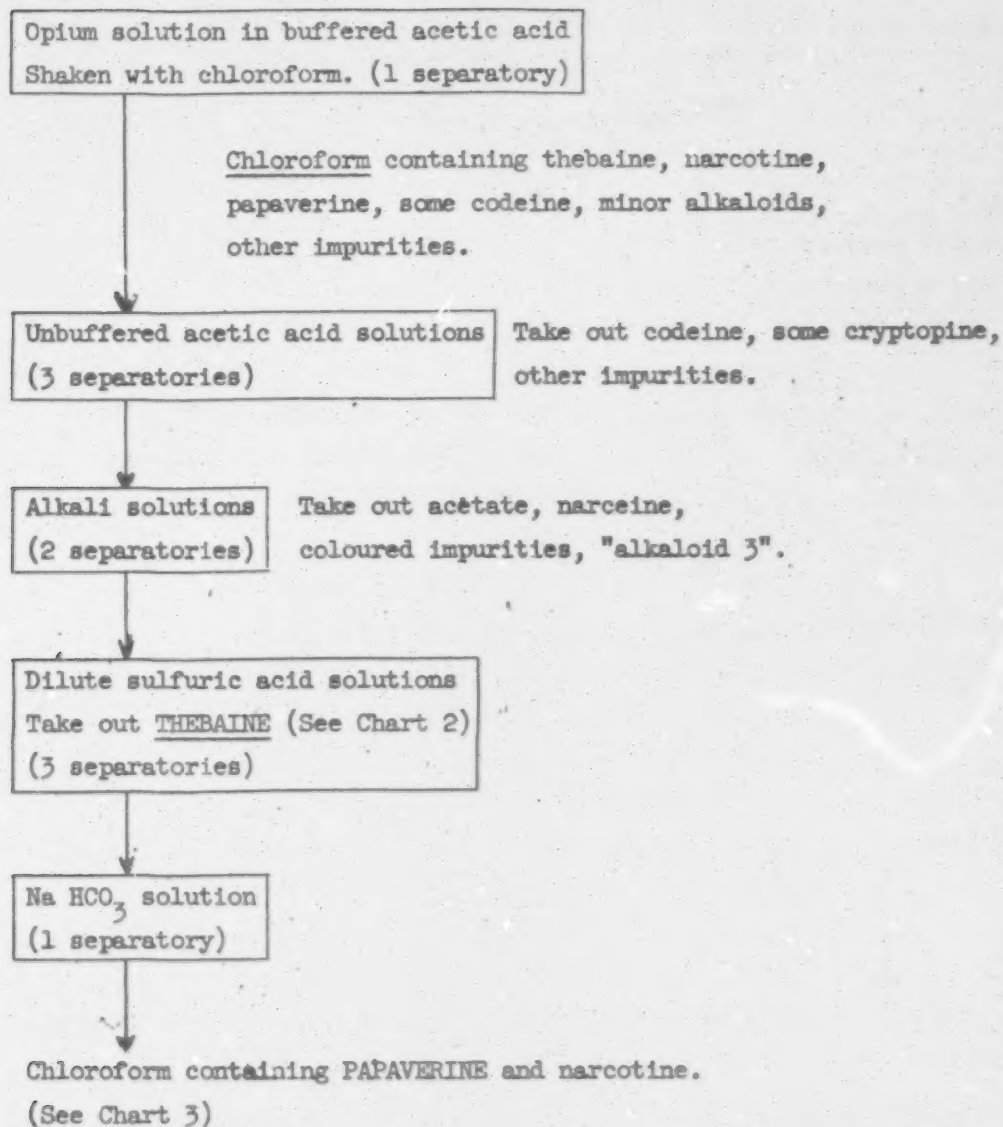


CHART 2

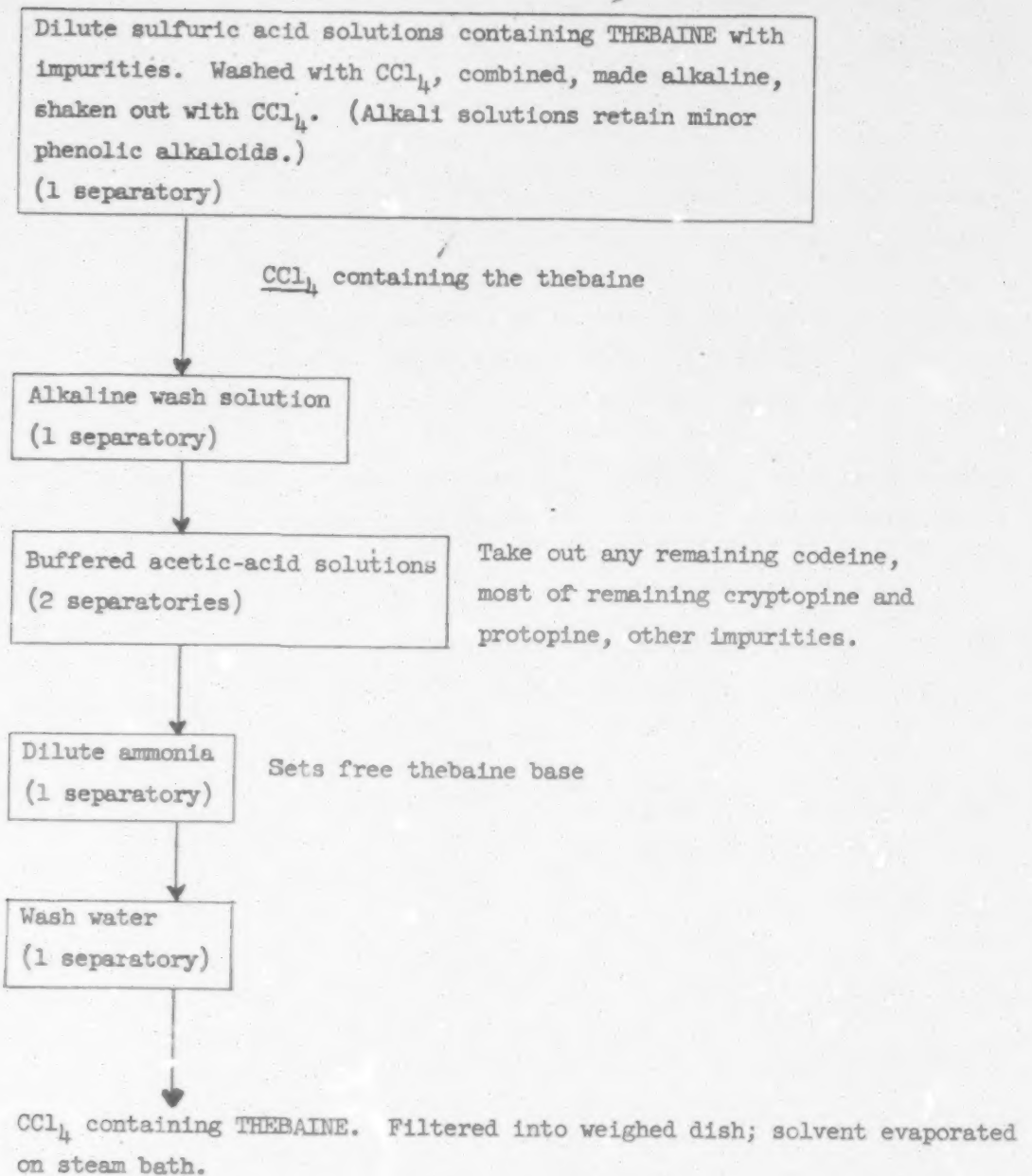
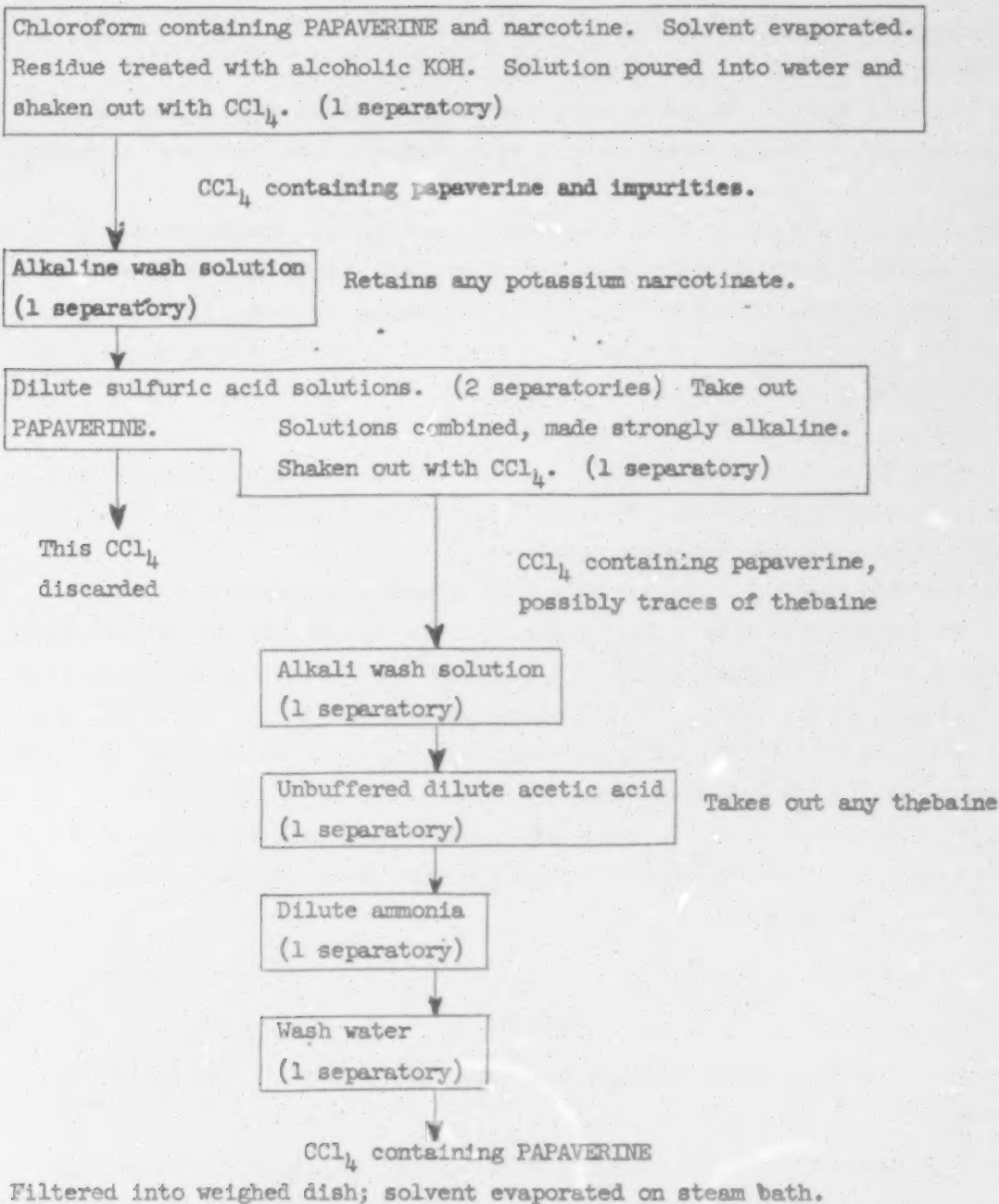


CHART 3



Reagents used for Colour Reactions in the Procedure

35. Concentrated HCl (orange with thebaine).
36. Syrupy H_3PO_4 (85%). (red-orange with thebaine)
37. (2 plus 1) H_2SO_4 . Two parts concentrated H_2SO_4 mixed with 1 part water. (orange-red with thebaine, same reaction with another -- an "unknown" -- substance in opium).
38. Buckingham's reagent. 10 to 15 gm ammonium molybdate dissolved in 100 cc of pure concentrated H_2SO_4 by heating on the steam bath. Keep in a well-stoppered bottle. (violet with protopine, green with cryptopine or codeine).
39. Molybdate- SO_3 reagent. A mixture of a few cc of Buckingham's reagent with an equal volume of fuming H_2SO_4 containing 15% free SO_3 . A new reagent. (purple with thebaine). Make up in small quantities.
40. Fuming Froehde's reagent. Mix 0.3 to 0.4 cc Buckingham's reagent with 4 or 5 cc fuming H_2SO_4 (15 to 25% free SO_3). (intense violet with papaverine). Use fairly fresh and test on known papaverine.
41. Persulfate in H_2SO_4 . About 0.2 to 0.25 gm ammonium persulfate per 10 cc concentrated H_2SO_4 . Must be fairly fresh, but the ingredients do not have to be weighed and measured accurately for ordinary tests. (orange-red with narcotine)
42. (3 plus 1) Marquis reagent. 4 drops formaldehyde solution (37 - 40%) mixed with 3 cc concentrated H_2SO_4 and 1 cc water. (purple with codeine but virtually negative to cryptopine and protopine). Use fairly fresh.
43. H_2SO_4 with ferric salt. 3 drops 10% $Fe_2(SO_4)_3$ solution with about 5 cc concentrated H_2SO_4 . (strong violet with cryptopine or protopine, virtually negative with codeine).

Caution

44. Carbon tetrachloride is required for some of the steps in the following procedure.

NOTE: IN CONSIDERATION OF THE DANGERS OF CARBON TETRACHLORIDE POISONING, BE SURE THE VENTILATION IS ADEQUATE AND AVOID, SO FAR AS POSSIBLE, BREATHING THE FUMES OF THIS SOLVENT.

Detailed Procedure for Thebaine and Papaverine in Opium

45. The following procedure requires about one day's working time to determine both thebaine and papaverine. It is not short, but neither is it tedious, as several series of different steps are required, each step having its own meaning. The description of the procedure is necessarily long when all the details are given.
46. Weigh 4.5 gm of the opium into a mortar having a pouring lip. Work up with 44 cc of 5% (by volume) acetic acid. Rub the opium marc and stir until solution is as complete as possible. Filter through a 12.5 cm folded filter paper. Take 30 cc of the filtrate (equivalent to 3 gm opium).

Isolation of Thebaine

47. Ten separatory funnels are temporarily necessary. These contain the following solutions:

- #1 - The opium solution, with the addition of 1 gm sodium acetate
- #2 - 8 cc of 5% acetic acid
- #3 - 6 cc of 5% acetic acid
- #4 - 5 cc of 5% acetic acid
- #5 - 20 cc of 10% NaOH solution
- #6 - 20 cc of 2 1/2% NaOH solution
- #7 - 14 cc of 1% (by volume) H_2SO_4
- #8 - 12 cc of 1% H_2SO_4
- #9 - 10 cc of 1% H_2SO_4
- #10 - 20 cc water containing a little $NaHCO_3$.

48. Begin the shakeout with 30 cc $CHCl_3$, and continue with 25 cc portions of $CHCl_3$. Each portion is shaken with the opium solution in separatory #1 and then with the other solutions in turn. (An emulsion forms with the opium solution but it will break after standing for some minutes.) From separatory #10 filter the $CHCl_3$ through a pledget of cotton into a flask for recovery of $CHCl_3$ and isolation of the papaverine therein.

49. Make four extractions of the original opium solution and move them along until the fourth extract has been tested, before starting another portion of $CHCl_3$ in separatory #1. Test the fourth extract from separatory #2 for the absence or

continued extraction of thebaine. Add a drop of ammonia to a little of the extract in a small porcelain crucible, evaporate on the steam bath, cool, and apply 3 or 4 drops of concentrated HCl. Thebaine yields an orange colour. A slightly delayed, diffused yellowish colour rather than a small spot which is distinctly orange before being dispersed in the solution, is indicative of the substance yielding a thebaine-like H_2SO_4 -reaction, rather than a trace of thebaine.

50. If no thebaine reaction is noted in this test, the contents of separatories #1 and #2 can both be discarded. If only a trace of thebaine is indicated, discard the original opium solution (separatory #1), but start a new portion of $CHCl_3$ with the solution in separatory #2, before discarding it. If thebaine is still coming out in more than a trace, continue the extraction of the original opium solution. If in doubt as to the nature of the test, repeat it on the same extract after shaking with the solution in separatory #3. If it is really due to thebaine, it will be as strong or stronger (from picking up some thebaine left from the preceding extracts); if the colour is only due to the other substance, it will be weaker on the test from separatory #3 (as some of this substance will be left in the aqueous layer). The thebaine should be all out of the original opium solution in four to seven extractions.

51. As soon as the original opium solution has been discarded, begin tests from separatory #5 (the first alkali solution). The acetic acid solutions do not hold any thebaine very long, usually only one extraction after the original opium solution is discarded. When only a trace of thebaine is found by HCl test, the $CHCl_3$ portion is passed on to the dilute H_2SO_4 solution in separatory #7, and the aqueous solutions in the preceding separatories are then discarded. If no thebaine is found in the extract tested from separatory #5, this $CHCl_3$ portion should not be passed on to separatory #7, but simply run into the $CHCl_3$ saved for redistillation, and the aqueous solutions in the separatories preceding then discarded.

52. The chloroform solutions already mentioned, processed to the end of the line of separatories, take with them most of the narcotine and papaverine, while leaving the thebaine in the H_2SO_4 solutions (separatories #7, #8, and #9). It now remains to shake out the last of the narcotine and papaverine from the H_2SO_4 solutions. (This may require as many as 6 or 8 additional extractions.) Start fresh portions of 25 - 20 cc $CHCl_3$ in separatory #7; make tests on the $CHCl_3$ from

separatory #9. Evaporate each test portion with a drop of ammonia, cool the crucibles, and test for narcotine with Persulfate in H_2SO_4 (orange-red colour indicates narcotine) and for papaverine with Fuming Frohde's reagent (papaverine yields an intense violet). Generally the papaverine reaction persists through more shakeouts than that of narcotine because it is more sensitive and the papaverine is harder to extract completely; but since some opiums contain virtually no papaverine, both tests should be tried at first.

53. Continue the extractions until the narcotine and papaverine reactions are absent or negligible. Then discard the $NaHCO_3$ solution. (The papaverine reaction is extremely sensitive, so only a small portion of $CHCl_3$ should be taken for this test, and any doubtful reaction should be disregarded. However if there is the least doubt of the strength of the fuming acid, try out the reagent on a tiny speck of known papaverine.) Run the $CHCl_3$ extracts into the same flask mentioned in paragraph 48.

54. Toward the end of the narcotine-papaverine extraction it may be advisable to test for the non-extraction of thebaine. At this stage, the test can be made with (2 plus 1) H_2SO_4 . A new sensitive reagent has been discovered which can also be used. The Molybdate- SO_3 reagent yields a purple colour with thebaine. It cannot be used to test for thebaine in the earlier stages of this procedure, as the unknown substance which yields a thebaine-like reaction with H_2SO_4 gives a strong dark colour, practically black. The Molybdate- SO_3 reagent does not contain enough fuming acid or free SO_3 to yield the Fuming Frohde's reaction with papaverine; papaverine yields a green colour but the reaction is much weaker than the intense violet with Fuming Frohde's. In case leakage of thebaine is noted, the last two H_2SO_4 solutions (in separatories #8 and #9) can each be poured back into the preceding separatory, and a fresh acid solution, 6 to 8 cc of 1% H_2SO_4 , put in separatory #9.

55. When the narcotine and papaverine have been removed from the three H_2SO_4 solutions, wash them with two successive portions of CCl_4 to remove residual chloroform. Discard the CCl_4 .

56. A new set-up of separatories is now made. The separatories #7, #8, and #9 now become #1, #2, and #3. Pour the acid solution of #2 into #1, and the acid solution of #3 into #2 and then into #1.

Separatory #1 now contains the combined acid solutions, and the following solutions are put into separatories #2 to #6:

#2 - 20 cc of 5% NaOH

#3 - 7 1/2 cc of 5% acetic acid, with 3 gm sodium acetate

#4 - 5 cc of 5% acetic acid, with 2 gm sodium acetate

#5 - 15 cc water plus 1 cc concentrated NH_4OH

#6 - 15 cc water.

57. Now make the combined acid solutions in separatory #1 alkaline with concentrated (1 plus 1) NaOH, and add 2 cc of the alkali solution in excess. Shake out with 30 - 25 cc portions of CCl_4 , shaking each portion with the other solutions in turn; however, leave the first portion, or if necessary the first two or three portions, in separatory #6 temporarily, to be run into an evaporating dish a little later.

58. Test the fourth shake-out from separatory #1 for continued extraction of thebaine. Evaporate a little of the CCl_4 in a small porcelain crucible and test the residue with syrupy H_3PO_4 . (In case of a doubtfully positive reaction the test can be checked by another test with concentrated HCl; but close the HCl bottle and remove all possibility of HCl fumes in the neighborhood before the CCl_4 extract of the thebaine is run into the final evaporation dish.) As soon as the thebaine is out of the solution in separatory #1 (generally in four extractions), discard it and also the alkali wash-solution in separatory #2.

59. It now remains to extract the last of the thebaine still in the buffered acetic-acid solutions. (This will require only a few more shake-outs.) Filter the CCl_4 from separatory #6 through a pledget of cotton in the stem of an ordinary funnel, into a weighed glass evaporating dish. A dish of 200 cc capacity is most convenient. Continue the extraction with portions of 20 - 15 cc CCl_4 .

60. Test the CCl_4 extracts from separatory #5; evaporate a small portion and test with H_3PO_4 . When the thebaine is out, discard the solutions in the separatories. Evaporate the collected solvent on the steam bath and heat until the residue is crystalline. Cool in the desiccator and weigh.

61. Dissolve the residue in a little methanol, add two or three drops methyl red indicator, and run in N/50 acid until the solution turns red. Add 2 or 3 cc excess and make sure that all the thebaine is in solution. Dilute with some recently boiled water. Back-titrate with N/50 NaOH, and then add N/50 acid again just to a faint acid colour. (If the N/50 alkali has not been made up fresh, and

is used but seldom, check its strength against the acid before computing the titration.) The cc of acid consumed, multiplied by 0.00622, gives the amount of thebaine. Multiply by 100 and divide by 3 for percentage.

62. The thebaine residue contains just a little protopine, but by this process it should be less than 0.1 per cent of the opium weight, and is probably not even enough to quite balance the slight losses of thebaine.

Determination of the non-thebaine alkaloids
in the titrated thebaine residue

63. The following procedure can be used for thebaine isolated by any method, to determine if it contains any considerable proportion of codeine, papaverine, cryptopine, or protopine. It is not used on all thebaine residues, but just often enough to test the method. With the above method, the isolated thebaine should contain no more than about 0.1% of the opium weight of other titratable alkaloids, mainly protopine.

64. Evaporate the titrated solution to about 5 cc (or to dryness on the steam bath, and take it up in 5 cc water). Pour into a small beaker or Erlenmeyer flask. Rinse in with 5 cc concentrated HCl and 5 cc water. Bring to boiling and keep near boiling temperature for about 5 minutes. This converts the thebaine to the phenolic thebenine.

65. Cool the solution and pour it into a separatory funnel. Rinse in with a little water. Add 1:1 NaOH solution gradually (with cooling if necessary) until strongly alkaline. In separatory #2 put an alkaline wash, in separatory #3 water with a very little ammonium salt and a drop of ammonia, and in separatory #4 water. Shake out with CCl_4 , shaking separatory #1 very gently, as a bad emulsion is easily formed, and allowing plenty of time for the emulsion to break. Shake the extracts with the solutions in the other separatories and filter into a weighed dish.

66. Completion of the extraction (in the case of thebaine isolated by the method of this paper) can be tested for with H_2SO_4 containing a little ferric salt (intense violet colour with cryptopine or protopine). (For tests useful for codeine and papaverine see paragraph 68 below.)

67. Evaporate off the CCl_4 on the steam bath, dry, cool, and weigh the residue.

Titrate with n/50 or n/100 acid. (A full titration will not be obtained in the ordinary way if the recovered alkaloid is papaverine, as it is a very weak base.)

68. After determining the amount of non-thebaine alkaloids, put a portion of the titrated solution into a separatory funnel and shake out the indicator from slightly acid solution with CCl_4 . Then make the solution alkaline or ammoniacal and shake with CCl_4 . Evaporate portions for tests. A test for codeine may be made with (3 plus 1) Marquis' reagent; for cryptopine-protopine with H_2SO_4 containing a little ferric salt. Buckingham's reagent yields violet with protopine, green with cryptopine (green also with codeine, but this is not so very sensitive). Papaverine may be tested for with Fuming Frohde's reagent in the absence of protopine and cryptopine (which will yield violet with Fuming H_2SO_4 alone). It could also be detected by micro-crystal test. A test with syrupy H_3PO_4 can be used to show that thebaine is no longer present.

Determination of Papaverine in the Chloroform from the
Thebaine Determination

69. Connect the flask containing the CHCl_3 with the narcotine and papaverine in it (paragraphs 48, 52-53) to a distilling condenser and distill off the CHCl_3 for reuse, down to about 50 cc (or less) remaining. Pour the chloroform solution into an ordinary evaporating dish, rinse in with a little CHCl_3 , and evaporate to dryness.
70. Put 4 pellets of KOH (about 0.4 gram) in a small Erlenmeyer flask with about 11 cc alcohol, cover with a small watch-glass, and warm on the steam bath until the KOH is dissolved. Then pour the alcoholic KOH on the papaverine-narcotine residue, stir it into solution with a glass rod, cover the dish with a watch-glass, and heat on the steam bath for a few minutes. Be sure that all the residue gets into solution and is heated.
71. Partially cool the alcoholic solution, and pour it into 10 cc water in separatory No. 1. Rinse in with 15 cc more water in portions. Rinse the dish also with 30 cc CCl_4 , pour this also into the separatory and shake.
72. The following separatories and aqueous solutions are used:
- #1 - the alkaline solution (paragraph 71) containing papaverine and potassium narcotinate
 - #2 - 25 cc wash water

#3 - 15 cc of 2% (by volume) H_2SO_4

#4 - 10 cc of 2% H_2SO_4

73. Shake the CCl_4 extract with each of the solutions in the separatories in turn, then reserve the solvent for redistillation. Shake thoroughly with the H_2SO_4 solutions and allow to settle well, as the papaverine is back-extracted into aqueous solution at this point. Continue the shakout with 25 - 20 cc CCl_4 at a time. Evaporate a small portion of the first CCl_4 from separatory #4 with a drop of ammonia and test the residue with Fuming Frohde's reagent for absence of papaverine. If necessary, add a third acid solution in a fifth separatory. Also test one or two subsequent portions of the CCl_4 , to be sure that all the papaverine remains in the acid solutions.

74. Test the 4th CCl_4 extract from separatory #1 for papaverine. (5 extractions at most should be enough.) The test is made with Fuming Frohde's reagent (paragraphs 40, 52, 53). When the papaverine is out of the starting solution, discard it and the wash solution (in separatories #1 and #2).

(Note: narcotine could easily be determined by reconvertng the potassium narcotinate. However, it is unlikely that 5% acetic acid, used for the original extraction of the opium, would dissolve all the narcotine. If the original extraction is made with plain water, the determination of the water-soluble narcotine of the opium at this point may be of interest.) Shake the CCl_4 past the acid solutions and reserve it for redistillation.

75. Then move the acid solutions to the first two positions. Pour the 2nd acid solution into the first. Put 4 cc rinse water into separatory #2 and make alkaline with a few drops NaOH solution, then pour into separatory #1. Put 20 cc water in separatory #2. Add three more separatories and solutions for the following set-up:

#1 - the combined acid solutions, containing papaverine

#2 - 20 cc wash water, slightly alkaline

#3 - 5 cc water plus 0.5 cc of 5% acetic acid

#4 - 20 cc water plus 1 cc concentrated NH_4OH

#5 - 20 cc wash water.

(The dilute acetic acid solution is precautionary, to take out any thebaine that may have got so far.)

76. Make the solution in separatory #1 alkaline with 10% or stronger NaOH and shake out with CCl_4 , using 25 cc at a time, until the papaverine is out of it, then discard it and the wash solution in separatory #2. Continue with 20 - 10 cc portions of CCl_4 until the papaverine is out of the dilute acetic acid solution.

77. Filter the CCl_4 extracts through a pledget of cotton into a weighed dish, evaporate off the solvent and heat on the steam bath until the whole residue has crystallized. (A little scratching and perhaps a trace of "seed" may help.) Cool in a desiccator and weigh. Multiply by 100 and divide by 3 for the percentage of papaverine in the opium.

Procedure for Papaverine alone

78. When it is not necessary to determine thebaine, the first part of the process, leading to the separation of narcotine and papaverine from thebaine, can be somewhat shortened.

79. Prepare a solution of the opium, using 5% acetic acid, as previously directed (paragraph 46). It is not necessary to buffer this solution with sodium acetate.

80. The separatories and solutions are as follows:

- #1 - the opium solution
- #2 - 20 cc of 10% NaOH solution
- #3 - 20 cc of 2 1/2% NaOH solution
- #4 - 10 cc of 2% H_2SO_4 plus 5 cc water
- #5 - 6 cc of 2% H_2SO_4 plus 6 cc water
- #6 - 5 cc of 2% H_2SO_4 plus 5 cc water
- #7 - 20 cc water plus a little NaHCO_3

81. Shake out with CHCl_3 , filtering the CHCl_3 from separatory #7 into an Erlenmeyer flask. Thebaine and other substances coming out will mask the papaverine reaction to colour reagents, but 5 shakeouts with CHCl_3 portions of 30 - 25 cc should be sufficient to remove all the papaverine from the original opium solution. Then discard the solutions in separatories #1, #2, and #3.

82. Continue the shakeout of the H_2SO_4 solutions with portions of 20 - 15 cc CHCl_3 , until the papaverine has been extracted. Test small portions from separatory #7 by evaporating the solvent and testing the residue with Fuming Frohde's reagent until the papaverine reaction is negative or insignificant. Then discard the aqueous solutions in the separatories.

83. Continue as previously described, paragraph 69 to 76, except that it may be best to use a slightly stronger acetic acid solution -- 5 cc water plus 1 cc of 5% acetic acid -- in the final shakeout of the papaverine (separatory #3, paragraph 74).

Results

84. A survey of the available samples is under way for their thebaine and papaverine values, but it will be a considerable time before it can be completed. Some representative results are given in Table I.

TABLE I.
Percentages of Thebaine and Papaverine in Opium

COUNTRY	THEBAINE	PAPAVERINE
Yugoslavia U.N. 38 a	1.64	2.42
Yugoslavia 63915 (1938 Export)	0.90	1.60
Yugoslavia, L. of N. V (1935)	0.92	1.99
Greece, U.N. 25 a	1.33	2.67
Turkey, U.N. 15, export type	1.42	1.44
Turkey, 44123 (1944 Export)	1.48	1.23
Turkey, seizure of Standardized opium	1.30	1.38
Iran, U.N. 20, Export	3.24	2.13
Iran, U.N. 47, Fars	4.10	2.12
Iran, sticks, blue green label	2.19	1.93
Afghanistan 63909 (morphine some 15%) (1943 Export)	2.10	0.22
Afghanistan 43533 (morphine some 10%) (1943 Export)	3.54	1.27
India, U.N. 34, Banaras	1.85	0.45
India, U.N. 35, Malwa	1.56	2.09
India, U.N. 36, Excise	1.76	0.66
India, 43385 (1944 Export)	2.30	2.26
Laos, U.N. 27B, Xieng Khouang	2.38	0.80
Vietnam, U.N. 28a, Laokay	4.85	0.10
China, U.N. 37B	1.40	0.84
China, 63423, Manchuria	1.07	1.23
Korea, 63431	2.18	0.65

Concluding Discussion

85. On looking at the method as a whole, it is easily seen that only minor changes would be necessary in order to make it possible to determine narcotine, codeine, and morphine, and perhaps also narceine, on the same sample of opium used for the determination of thebaine and papaverine. These determinations would, however, require a number of changes in the details of the procedure. It is intended to work them out in the future.

86. The nature of the substance yielding a sulfuric-acid reaction similar to that of thebaine has not been established. Further investigation of this substance will be made as soon as time permits.

87. It is of interest that the analysis of a few samples has shown virtually no papaverine (see paragraph 33). This has been reported at least three times before:

(a) L. van Itallie and M. Kerbosch, in examining qualitatively 19 samples of opium from seven different regions of the world for the six principal alkaloids, found all six in all of them except that 4 of the 5 samples from India contained no detectable papaverine. ("Beitrage sur Zusammensetzung des Opiums". Archiv der Pharmazie 248,609-613 (1910).)

(b) K. A. Chassovnikova reported that opium from a certain Chinese variety of Papaver somniferum contained neither papaverine nor narcotine. (Biokhimiya 2, 701-704 (1937).)

(c) A. H. Homeyer at Mallinckrodt Chemical Works, St. Louis, U.S.A., in a personal communication a few years ago reported some Afghan opium as containing less than 0.1 per cent papaverine (by his own method of analysis). This is the same type of Afghan opium as found by the Secretariat to contain virtually no papaverine. (The result of the analysis is shown in Table I as 0.22% by weighing, but this residue failed to give any distinct qualitative indications of true papaverine.)

88. The thebaine and papaverine percentages thus far determined do not as yet clearly show any broad geographical classification. Instead, they often reveal subgroups. For example, the two types of opium from Afghanistan, though similar in codeine and porphyroxine content and microscopically, are distinctly different in thebaine and papaverine content as well as morphine percentage. In Turkish opiums, some wide variations have been found from the values for export samples as given in Table I. It should not be surprising that sub-groups in the opium

classification are found, since such producing countries as India and Turkey each recognize different types of opium in the domestic production.

89. At present the use of thebaine and papaverine values for determination of the origin of seized opium seems to lie chiefly in a matching technique: i.e., showing that a seizure corresponds in type -- in general and microscopical appearances and in composition-relations -- with a sample of opium known to originate in a certain country, and that it differs in some essential respects from the opiums known to originate elsewhere. The composition-relations are being worked out at present on the basis of "curves" indicating the morphine, codeine, thebaine, papaverine, and porphyroxine values for each sample.

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THE ASSAY, CHARACTERISTICS, COMPOSITION, AND ORIGIN OF OPIUM*

No. 18

The assay of morphine in opium, and determination of the total
of minor phenolic alkaloids

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In continuation of the international programme of scientific research undertaken pursuant to resolutions 159 II C (VII) and 246 F (IX), adopted by the Economic and Social Council on 3 August 1948 and 6 July 1949 respectively, the Secretary-General is presenting the following paper on the assay of morphine in opium, from the laboratory of the Narcotics Bureau of the Republic of China. The method of assay is designed to make it possible to determine the important non-phenolic alkaloids of opium on the same sample used for the morphine assay. In addition, it yields a figure for the percentage of the minor phenolic alkaloids, which will be of considerable interest to opium chemists.

* Inclusion of an article by contributing scientists in this series does not necessarily mean that the Secretariat shares the views expressed.

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THE ASSAY OF MORPHINE IN OPIUM,
AND DETERMINATION OF THE TOTAL
OF MINOR PHENOLIC ALKALOIDS

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Introduction

1. The purpose of this paper is to present an extraction method for the assay of morphine in opium, designed to provide for the possible determination of the important non-phenolic alkaloids on the same sample.
2. For this reason the opium is extracted with dilute acid, instead of lime water, and then the solution is made alkaline and the non-phenolic alkaloids are extracted with ether.
3. In preliminary work on the method, no dichloroacetic acid was available to the Taiwan Narcotics Laboratory, and the attempt was made to remove minor phenolic alkaloids by two steps: filtration of the solution buffered to pH 6, and extraction of the acidic buffered solution with carbon tetrachloride. The latter step was carried out to remove porphyroxinemeconidine, and doubtless some similar alkaloids with it. However, it appears that not all the minor phenolic alkaloids were removed by these steps.
4. When dichloroacetic acid became available to the Taiwan Laboratory, the method of separating the other phenolic alkaloids from morphine, essentially as given in the Secretariat's document ST/SCA/SER.K/12, was found of most value, and has been incorporated in the present method, in place of the two steps mentioned in paragraph 3.

5. Besides the assay for morphine content, and separation of the non-phenolic alkaloids, the method now yields a determination of the total of minor phenolic alkaloids not extractable by ether from alkaline solution. From the few results already obtained it appears that this figure is sometimes less than one per cent, but occasionally even above two per cent.

Summary of the assay

6. A weighed opium sample is extracted with dilute sulfuric acid in order to get all the alkaloids into solution.

7. An aliquot portion of the filtered acidic opium solution is rendered alkaline with sodium hydroxide, treated with a small amount of potassium sulfite, and extracted with ether, in order to remove non-phenolic alkaloids (principally papaverine, narcotine, thebaine, cryptopine, and codeine).

8. The alkaline solution is neutralized with sulfuric acid to litmus and concentrated to a small volume. A definite amount of dichloroacetic acid is added and the solution extracted with chloroform in order to remove phenolic alkaloids other than morphine.

9. The acid solution is then rendered ammoniacal with ammonia water in excess and extracted with chloroform-alcohol mixture. Morphine is determined by titrating with standard sulfuric acid.

10. The purity of the assay morphine is then tested according to the method described in document ST/30A/SER.K/12.

Experimental

A. The assay:

11. Weigh accurately 3 grammes of air-dried raw opium, triturate in a mortar with 5 cc of 0.1 N sulfuric acid to a smooth paste and then with 24.4 cc more of the same acid. Continue the trituration for half an hour in order to make a complete solution of all the opium alkaloids.
12. Filter the mixture and transfer with a pipet 20 cc of the filtrate, representing 2 grammes of dried opium, to a light-liquid continuous extraction apparatus. Add 3 cc of 3 N sodium hydroxide and 0.1 grammes of potassium sulfite, and extract with pure ether. After 3 hours of extraction, test the residue obtained by evaporating 1 cc of the ether taken from the side tube of the extraction apparatus for no reaction of codeine with Marquis' reagent.
13. When the extraction is complete, transfer the whole content of the extraction column to a separatory funnel (not including the ether extract in the receiving flask), with the necessary precaution of washing thoroughly the extraction column with small portions of water and adding to the separatory funnel. Separate the alkaline solution from the ether layer into a dish. The ether is then washed with 2 cc of 0.5 N sodium hydroxide and with 2 cc portions of water until the residue, obtained by evaporating 5 drops of the last washing, gives a negative reaction for morphine with Marquis' reagent. Add the washings to the main aqueous solution in the dish. Combine the washed ether with the main ether extract in the receiving flask and reserve the ether extract for the determination of papaverine, narcotine, thebaine, cryptopine, and codeine. (This determination will be described separately.)
14. Neutralize the alkaline solution in the dish with 1 N sulfuric acid to litmus and concentrate the mixture on the water-bath to about 10 cc.
15. Transfer the solution to separatory funnel No. 1, wash the dish thoroughly with small portions of water and add the washings to the separatory funnel. Add 0.4 cc dichloroacetic acid and extract the solution with chloroform.

Transfer the CHCl_3 with shaking through the following solutions: in separatory No.2 - 7.5 cc water plus 0.1 cc dichloroacetic acid; No.3 - 5 cc water; No.4 - 10 cc water plus 10 drops of NH_4OH ; No.5 - 10 cc water. In transferring the CHCl_3 from separatory No.1 to No.2, filter through a pledget of cotton in a short-stemmed funnel. Finally filter the CHCl_3 from separatory No.5 through a pledget of cotton into a flask. Occasionally test the residue from a little of the CHCl_3 extract with Froehde's reagent (green, then violet). Continue the extractions with 20 cc portions of CHCl_3 until the CHCl_3 drawn from separatory No.5 gives no more reaction with Froehde's reagent. (Be sure that no morphine reaction appears).

16. Combine the acid solutions by pouring from the second separatory into the first, and from the third into the second and then into the first. Then put 5 cc water in the third separatory, shake and pour it into the second. Put again 5 cc water in the third separatory, make it ammoniacal with 1-2 drops of NH_4OH and shake, then pour it into the second. Finally add 5 cc water to the third separatory.

17. Add ammonia water to the first separatory funnel to render the aqueous solution basic to litmus with ammonia water, add 1 cc in excess, and extract with chloroform-alcohol mixture (80:20). Transfer the solvent into the second separatory, shake and transfer it to the third. Shake and transfer the solvent into a flask. Continue the extractions with portions of chloroform-alcohol mixture, until 5 drops of the last solvent extract after evaporating off gives no morphine reaction with Marquis' reagent.

18. Distil off the solvent and dry at 100°C for 15 minutes. Add from a buret a definite quantity of standard 0.1 N sulfuric acid into the flask and warm on the water-bath to ensure complete solution of the base. Titrate back with 0.1 N sodium hydroxide, using methyl red indicator. The number of cc of the 0.1 N acid consumed multiplied by 0.02853 gives the weight of morphine in 2 g of opium.

19. Distil off the chloroform from the extract of paragraph 15, and dissolve the residue in a few cc of 0.02 N sulfuric acid from a buret, add methyl red indicator, titrate back with 0.02 N sodium hydroxide, and calculate the minor phenolic alkaloids in terms of morphine.

Discussion

24. Dilute sulfuric acid is used for preparing the solution of total opium alkaloids instead of lime water, for the acid solution probably contains all the alkaloids of opium without any appreciable loss: thus a systematic determination for all the important alkaloids in opium will be made possible on one sample, whereas the lime solution dissolves quantitatively only codeine and some phenolic alkaloids, besides morphine. This is rather important in examining a small sample of opium as completely as possible.

25. The total volume of dilute sulfuric acid is made to total 29.4 cc. Taking into consideration the volume produced by the extractives in opium, which is taken as about 0.2 cc per gramme of opium, the total volume of the final acid solution is assumed to be 30 cc.

26. Ether is used to extract all narcotine, papaverine, thebaine, cryptopine, and codeine from the sodium hydroxide solution of opium with no loss of morphine, so that a complete determination of the above alkaloids can be made as part of the same procedure. The preliminary removal of these non-phenolic alkaloids, especially codeine, renders the subsequent extraction of the dichloroacetic acid solution with CHCl_3 more complete, for codeine is far more difficult to remove than the minor phenolic alkaloids in this way.

27. Potassium sulfite is used to prevent the **oxidation** of morphine during the extraction of the alkaline solution with ether.

28. The separation of the minor phenolic alkaloids is carried out by the extraction of the opium solution containing dichloroacetic acid with chloroform according to the pattern of the method given in document ST/SCA/SER.K/12.

29. The percentages found for minor phenolic alkaloids differ considerably with different samples, and are much higher than those reported for minor phenolic alkaloid in ST/SCA/SER.K/12. It should be noted that the method of the United Nations Secretariat described in the document cited yields only the value for minor phenolic alkaloid not extractable by chloroform from alkali, whereas the present method yields the total of minor phenolic alkaloids not extractable by ether from alkali. This probably explains the difference, since chloroform is known to extract some of the minor phenolic alkaloids from alkali rather readily; but it is also possible that the acid extraction of opium may yield a greater total of such alkaloids than the lime method.

B. Confirmation test for the purity of the assay morphine:

20. Transfer the titrated solution of morphine in the assay to a separatory funnel, wash the flask thoroughly with small portions of water, add the washings to the separatory, add 3 cc of 3 N sodium hydroxide and extract with ether. Test the extract for codeine, thebaine, papaverine, cryptopine, and narcotine with the different appropriate reagents.
21. Separate the aqueous solution from the ether, transferring it into a second separatory funnel. Wash the ether with 2 cc of water and add the washing to the aqueous solution in the second separatory funnel. Acidify the solution with 1 M phosphoric acid, extract with 2-3 portions of CCl_4 in order to remove the indicator.
22. Render the aqueous solution in the second separatory funnel basic to litmus with an excess of NaHCO_3 and extract with chloroform. Transfer the CHCl_3 with shaking through the following solutions: in separatory No. 3 - 10 cc water plus 0.4 cc dichloroacetic acid; No. 4 - 7.5 cc water plus 0.1 cc dichloroacetic acid; No. 5 - 5 cc water; No. 6 - 10 cc water plus 10 drops concentrated NH_4OH ; No. 7 - 10 cc water. In transferring the CHCl_3 from separatory No. 3 to No. 4, filter it through a pledget of cotton in a short-stemmed funnel. Finally filter the CHCl_3 from separatory No. 7 through a pledget of cotton into a flask. Test the CHCl_3 extract with Froehde's reagent. If any non-morphine reaction is noted, continue the extractions with 20 cc portions of CHCl_3 , and determine the minor phenolic alkaloids as in paragraph 19.

Some results

23. The following are a few results obtained by the Assay on some U. N. samples of opium:

Opium sample	Assay		Confirmation test	
	Total phenolic minor alkaloids* (per cent)	Morphine (per cent)	Non-phenolic alkaloids in morphine	Other phenolic alkaloids in morphine
U.N.377 China, A	0.52	10.82	0	0
U.N.35 India, Malwa	0.70	12.21	0	0
U.N.26 Indo-China, Luang Prabang	0.57	11.58	0	0
U.N.7 Turkey, Merzifon, C.	2.15	20.80	0	0
U.N.38 Yugoslavia, No.2	0.53	18.28	0	0

*Calculated in terms of morphine.

30. The assay morphine, tested by the procedures described under paragraphs 20 to 22 inclusive, was found to be entirely free not only from non-phenolic alkaloids but also free from other phenolic alkaloids, so far as the method of dichloroacetic acid is concerned. This shows again that preliminary removal of the non-phenolic alkaloids, before the dichloroacetic acid separation, is preferable.
